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Effect of Repeated Skin Application on Percutaneous Absorption of Salicylic Acid

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Abstract □ Various concentrations of salicylic acid in hydrophilic ointment were applied repeatedly at daily or weekly intervals to rats *in vivo*. Salicylic acid absorption through treated skin was monitored by determining the penetration fluxes of salicylic acid through skin excised at various times. A gradual decrease in the salicylic acid penetration flux was observed following weekly applications of either 5 or 10% salicylic acid in hydrophilic ointment. The penetration flux of 1% salicylic acid remained constant. In the daily applications of 5 and 10% salicylic acid, the penetration flux increased after approximately 2 days of treatment and declined thereafter. The penetration flux of salicylic acid from the 1% salicylic acid increased slightly after 3–4 days of treatment.

Keyphrases □ Salicylic acid—percutaneous absorption, effect of repeated application, rats □ Absorption, percutaneous—salicylic acid, effect of repeated application, rats □ Keratolytic agents—salicylic acid, percutaneous absorption, effect of repeated application, rats

The percutaneous absorption of salicylates has been reported extensively (1–5). Barry *et al.* (6) examined blood salicylate levels in rabbits treated with 10% salicylic acid in hydrophilic ointment at weekly intervals. They reported that a progressive and statistically significant decrease in percutaneous absorption of salicylic acid occurred over the treatment period. Skin dehydration and decreased emotional arousal of the animals were suggested as possible explanations for this result.

BACKGROUND

Since salicylic acid is usually applied to the skin in topical therapy with repeated applications, it was decided to evaluate the effect of repeated applications of salicylic acid on its percutaneous absorption. Blood salicylate levels resulting from salicylic acid absorption following repeated topical applications (6) may reflect not only changes in absorption rates due to the dermatological effects of salicylic acid but also other pharmacokinetic alterations in metabolism and excretion due to the prolonged therapy.

Müller *et al.* (7) reported decreased steady-state plasma salicylic acid levels associated with chronic aspirin ingestion. Percutaneous absorption of substances was examined using excised skin (8, 9). Excised skin allows intracutaneous penetration to be measured simply, directly, reproducibly, and precisely, the fundamental assumption being that the stratum corneum is a dead tissue whose impermeability is unaffected by excision. The use of skin excised following repeated applications of salicylic acid to the skin *in vivo* was employed in the present study to examine directly the consequences of the dermatological effects of salicylic acid on its absorption.

The wide range of dermatotherapeutic effects of salicylic acid is also concentration dependent (10). In concentrations of 5% or more, salicylic acid exerts a keratolytic effect on the skin; in concentrations of less than 5%, a keratoplastic effect is apparent (10). Consequently, the objective of this study was to assess: (*a*) the effect of daily or weekly repeated topical applications of salicylic acid on its absorption, and (*b*) the effect of the concentration of salicylic acid in the hydrophilic ointment on its absorption.

EXPERIMENTAL

Animals—Female Wistar rats[1], 250–300 g, were housed in a constant-temperature room and given food[2] and water *ad libitum*.

Ointment Base—Salicylic acid BP was passed through an 80-mesh sieve, and appropriate amounts were incorporated into hydrophilic ointment USP. Accordingly, hydrophilic ointments containing 10, 5, and 1% salicylic acid were prepared.

Test Procedures—Under light ether anesthesia (anesthetic ether BP), the rats were weighed and hair was removed from the flanks with an animal clipper[3]. A sample of ointment was rubbed into the area of shaved skin; this area (3 cm^2) was approximately twice the cross-sectional area for diffusion in the diffusion cells. Additional ointment (total ~2 g) was spread uniformly over the area on the dull side of a sheet of aluminum foil. The foil was held in place by adhesive tape and an elastic bandage.

The animals were kept in restraining cages for the duration of the treatment. After the 7.5-hr test period, the bandages were removed from the animals and the animals were returned to the animal house. Repeated treatments at daily or weekly intervals were then employed. At various stages during the treatment, the animals were asphyxiated by an overdose of ether, the skin immediately was excised, and the appropriate ointment was applied to the epidermis. The treated excised skin was placed in a diffusion cell (9); the receptor compartment contained 0.1% chlorhexidine solution to prevent microbial growth at 37°.

To measure the amount of salicylic acid penetrating the skin, 3-ml aliquots of receptor solution were removed and treated with 1 ml of 13.2% ferric chloride in 0.4 *N* HCl. The resultant color was measured[4] at 540 nm, and the salicylate concentration was computed from a Beer's law plot. The 3-ml aliquot of solution removed from the diffusion cell was replaced with 3 ml of the chlorhexidine solution. A minimum of three animals was used in the estimation of each reported penetration flux. The possible interaction between salicylic acid and the skin on the initial application was examined by comparing the fluxes of various concentrations of salicylic acid in hydrophilic ointment to its flux across an inert membrane

[1] Southern Biological Services, Parkdale, Victoria.
[2] M & V Mouse Cubes, W. M. Charlick, Adelaide, South Australia.
[3] Breville model 900.
[4] Beckman spectrophotometer.

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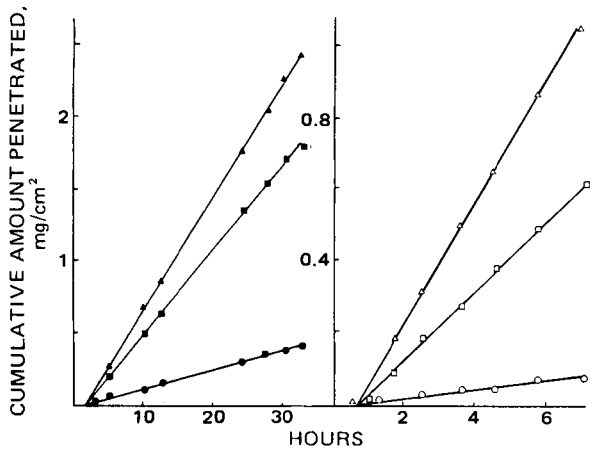

Figure 1—*Typical plots of cumulative amount of salicylic acid penetrating through excised rat skin (left) and dimethyl polysiloxane membranes (right) with time. Key: ●○, 1% salicylic acid in hydrophilic ointment; ■□, 5% salicylic acid in hydrophilic ointment; and ▲△, 10% salicylic acid in hydrophilic ointment.*

(dimethyl polysiloxane[5]) according to the method of Roberts and Anderson (9).

Thick sections for light microscopic observations were prepared by the method of Roberts *et al.* (8), except that Spurs resin[6] was used as the embedding medium.

RESULTS AND DISCUSSION

The relation between the cumulative amount of salicylic acid penetrating through single samples of excised skin and dimethyl polysiloxane with time following a single application is shown in Fig. 1. Similar plots were observed for repeated applications. After a lag time, the plot of the cumulative amount of solute penetrating against time was linear, the slope of this line being the steady-state penetration flux.

Table I shows the mean penetration fluxes observed through dimethyl polysiloxane and excised skin with the ratio of the fluxes. The ratio of fluxes decreased with increasing concentrations of salicylic acid. This result may be ascribed to the suspended salicylic acid particles reducing the occlusive properties of the hydrophilic ointment or to a dehydrating effect of the salicylic acid. However, Loveday (2) showed that the rate of penetration of salicylic acid from aqueous solutions (pH 2.2) through excised pig skin was directly proportional to the concentration of salicylic acid used. The decreased ratio suggests that a single application of salicylic acid does not damage the skin significantly. Marked increases in the ratio of fluxes (skin to inert membrane) were observed when high damaging concentrations of phenol were applied to the skin (8, 9).

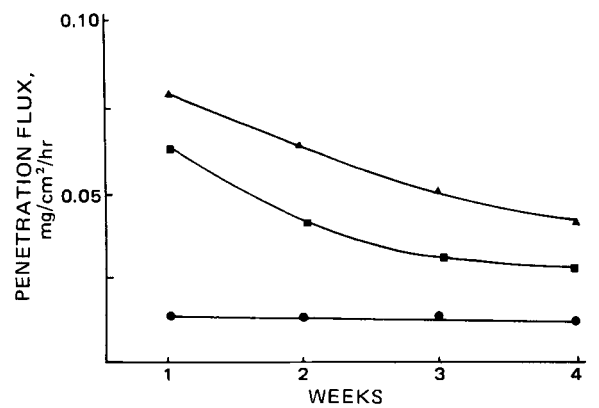

Figure 2—*Mean penetration fluxes of salicylic acid through excised rat skin following weekly treatments with salicylic acid* in vivo. *Key: ●, 1% salicylic acid in hydrophilic ointment; ■, 5% salicylic acid in hydrophilic ointment; and ▲, 10% salicylic acid in hydrophilic ointment.*

[5] Dow Corning Silastic sheeting 501-1.
[6] Ladd Chemicals, New York, N.Y.

Table I—Mean Penetration Fluxes (±*SE*) of Salicylic Acid in Hydrophilic Ointment Base through Dimethyl Polysiloxane Membranes and Excised Rat Skin following a Single Treatment

Salicylic Acid Concentration (w/w)	Penetration Flux of Salicylic Acid (± *SE*), mg/cm²/hr		Ratio of Fluxes: Skin–Dimethyl Polysiloxane
	Excised Skin	Dimethyl Polysiloxane	
1	0.014 ± 0.002	0.016 ± 0.001	0.88
5	0.061 ± 0.003	0.099 ± 0.001	0.62
10	0.078 ± 0.003	0.159 ± 0.001	0.49

The mean penetration fluxes of salicylic acid through rat skin following repeated weekly applications of 5 and 10% salicylic acid in hydrophilic ointment over 4 weeks of treatment are shown in Fig. 2. An analysis of variance of the penetration fluxes observed for each week of treatment revealed a significant difference between the treatments with both the 5% ($F = 58.6, df = 3/12, p < 0.001$) and 10% ($F = 9.97, df = 3/12, p < 0.01$) concentrations. The decline in fluxes paralleled the reduction in blood levels observed when rabbits were treated with 10% salicylic acid in hydrophilic ointment at weekly intervals (6). These results suggest that the reduction in fluxes or blood levels may be attributed directly to the local effects of the salicylic acid. The treatment with 1% salicylic acid in hydrophilic ointment did not alter the salicylic acid penetration flux. The relative changes in fluxes produced by the 5 and 10% salicylic acid ointments were similar.

Figure 3 shows the mean penetration fluxes of salicylic acid following repeated daily applications. An analysis of variance of the penetration fluxes observed for each day of treatment revealed a statistically significant difference between the treatments of all concentrations (1%, $F = 8.87, df = 4/10, p < 0.01$; 5%, $F = 20.2, df = 4/10, p < 0.001$; and 10%, $F = 4.14, df = 4/10, p < 0.05$).

For the higher concentrations of salicylic acid, an increase in penetration flux was observed after 2 days. Following further treatment, this flux gradually decreased. The increase in flux was quite substantial and supports reports that treatment of the skin with salicylic acid may facilitate the absorption of other medicaments (10). Daily treatment with 1% salicylic acid in hydrophilic ointment also increased flux, but this flux was not readily apparent before 3–4 days.

The histological changes resulting from the topical application of ointments containing salicylic acid were reported previously (11, 12). The histological changes to the rat skin following treatment with salicylic acid preparations in this study were consistent with the observations of Strakosch (11), who observed that the keratolytic changes began after 2–3 days of treatment with 5 or 10% salicylic acid in an oxycholesterol–petrolatum base and after 7 days of treatment with 1% salicylic acid in this base.

The initial increases in the penetration flux observed for daily treatment with 1, 5, and 10% salicylic acid in hydrophilic ointment (Fig. 3) corresponded to the histological observations of swelling and exfoliation. The times for keratolysis implicit from the penetration fluxes and his-

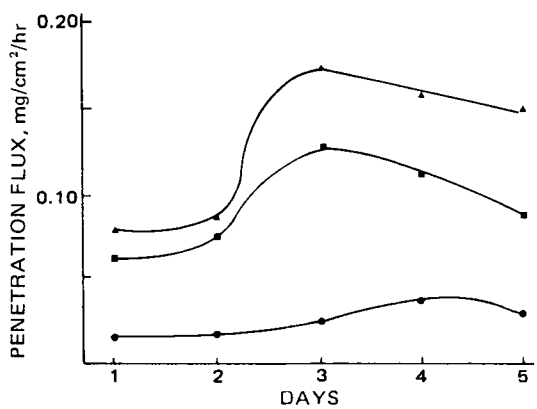

Figure 3—*Mean penetration fluxes of salicylic acid through excised rat skin following daily treatments with salicylic acid* in vivo. *Key: ●, 1% salicylic acid in hydrophilic ointment; ■, 5% salicylic acid in hydrophilic ointment; and ▲, 10% salicylic acid in hydrophilic ointment.*

tological changes in this study were shorter than those reported by Strakosch (11). These differences may be ascribed to the use of rat rather than human skin or different base formulations. Strakosch also found that the onset of keratolysis was markedly dependent on the base used, the onset being two to three times more rapid for salicylic acid in the oxycholesterol–petrolatum base than in the petrolatum base. Since higher blood salicylic acid levels were observed from the topical application of salicylic acid in a hydrophilic ointment base compared to a petrolatum base (1), keratolysis apparently depends on the absorption rate of the salicylic acid from the particular formulation.

Following the initial keratolytic process in which morphological changes were observed in the stratum spinosum, stratum granulosum, and stratum corneum, further repeated applications of salicylic acid gave rise to the formation of a broad stratum corneum. The process by which this broad stratum corneum formed was not clearly evident but may have resulted from diminished fluxes after prolonged daily (Fig. 3) or weekly (Fig. 2) treatments.

Since the salicylic acid being applied still exerts its surface keratolytic effect, reduced absorption *in vivo* may be due not only to the thicker horny layer but also to the process of desquamation. The results of this study, using an *in vitro* diffusion apparatus, suggest that the thicker horny layer does offer greater resistance to the penetration of salicylic acid.

Recent work by Davies and Marks (12) did not substantiate the increased epidermopoiesis resulting from the application of salicylic acid to human skin as described by Strakosch (11). Davies and Marks did observe differences in the horny layer structure and thickness in treated and untreated samples of skin. As stated by Davies and Marks (12), it is possible that their experimental conditions were not sufficiently severe or of a sufficient duration for increased epidermopoiesis to be observed.

Although the present histological and physiochemical results are consistent with a dynamic equilibrium between keratolysis and regeneration of the epidermis, the precise cytological changes in the epidermis following salicylic acid therapy have yet to be fully documented. The changes resulting from the present experimental conditions are being examined using transmission electron microscopy.

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ESTRIP, a BASIC Computer Program for Obtaining Initial Polyexponential Parameter Estimates

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Received January 30, 1978, from the *Department of Pharmacology and Therapeutics, Louisiana State University School of Medicine in Shreveport, Shreveport, LA 71130.* Accepted for publication April 6, 1978.

Abstract □ A new BASIC exponential stripping program, ESTRIP, allows the relatively rapid calculation of initial polyexponential parameter estimates, as does the previously published FORTRAN IV program, CSTRIP. The potential advantages of the new program are that it can be run on microcomputers and minicomputers with BASIC capability and a relatively small core and that it can be easily modified by the user.

Keyphrases □ Computer programs—ESTRIP for calculation of initial polyexponential parameter estimates □ Pharmacokinetic models—ESTRIP computer program for calculation of initial polyexponential parameter estimates □ Models, pharmacokinetic—ESTRIP computer program for calculation of initial polyexponential parameter estimates

The mathematical solutions of classical linear pharmacokinetic models are given by the sums of exponential terms. The generalized equation for these models can be written as:

$$C = \sum_{i=1}^{u} a_i e^{-b_i t} \qquad \text{(Eq. 1)}$$

where C is the concentration of drug at time t, u is the number of exponential terms, and a_i and b_i are the parameters to be determined.

Computer programs for the estimation of these polyexponential parameters (1, 2) generally require sophisticated computers utilizing BMD, BMDP, SAS, or other expensive software packages and are usually available on a time-sharing, batch-job basis. One exception is the CSTRIP program (3), which can be used to obtain preliminary polyexponential parameter estimates *via* an automated stripping (feathering, peeling-off, or back-projection) technique; this program requires the use of FORTRAN IV.

The purposes of this paper are to describe a new exponential stripping computer program[1] and to illustrate its use. The potential advantages of the new program are that

[1] A complete photocopy of the program listing will be supplied upon request. The program is also available, for a fee, on paper tape or a Scotch DC300A Data Cartridge.

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