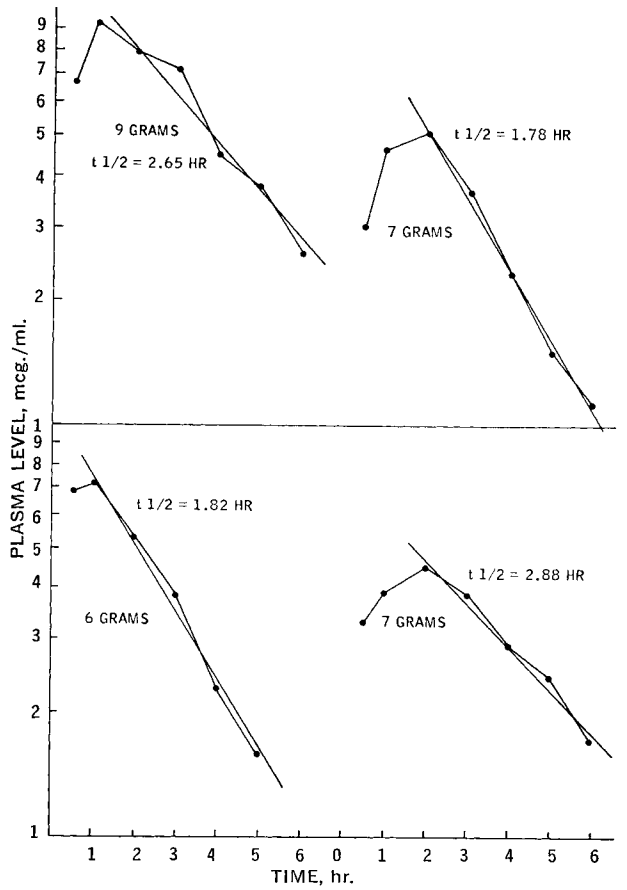

Figure 5—*Calculated disappearance curves and blood level data of plasma oxime levels in several subjects.*

mucosa) rather than to a systemic effect. This degree of side effects would probably preclude chronic administration.

SUMMARY AND CONCLUSIONS

Single and repeated doses of a commercially available tablet of 2-PAMCl were given to volunteers. Five grams was required to produce a plasma level of 4 mcg./ml. after a single dose, and higher single doses (to 9 g.) produced plasma levels up to 11 mcg./ml. However, there was much variation in blood levels among individuals receiving the same dose. Although it was possible to maintain blood levels of more than 4 mcg./ml. when the oxime was given in doses higher than 3 g. at 4-hr. intervals, all subjects who received multiple doses over 48 hr. had gastrointestinal symptoms. The mean urinary excretion was 20–25% of the dose administered; the plasma $t_{0.5}$ was 2.7 hr. and the $t_{0.5}$ for excretion into the urine was 2.4 hr.

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Enhancement of Percutaneous Absorption by the Use of Volatile: Nonvolatile Systems as Vehicles

M. F. COLDMAN, B. J. POULSEN, and T. HIGUCHI

Abstract □ Using *in vitro* techniques the penetration of ^{14}C labeled fluocinolone acetonide and its acetate ester through human skin at 37° has been examined with vehicle mixtures of isopropanol and isopropyl myristate or propylene glycol. Little penetration was found from either of the nonvolatile solvents. As the formulation was changed to include increasing amounts of volatile component, however, penetration could be increased up to 8 to 10 times. Precipitation of steroid prevented greater increases.

Keyphrases □ Percutaneous absorption—enhancement □ Volatile: nonvolatile vehicles—percutaneous absorption □ Fluocinolone acetonide and fluocinolide, ^{14}C-labeled—percutaneous absorption □ Scintillometry—analysis □ Radiochromatography—analysis

The penetration of corticosteroids through the intact human epidermis has been shown to be very poor (1, 2) and occlusive techniques are commonly used to increase penetration (3, 4). However, these methods are neither convenient nor pleasing to the patient and this investigation has studied another means of enhancing penetration that may make occlusion unnecessary.

Based on theoretical models, Higuchi (5) derived equations outlining parameters of percutaneous absorption and other workers (6–8) have investigated some aspects of vehicle effects on the degree of penetration of topically applied drugs. This report is primarily concerned with the effect, on penetration, of concentrating the drug into a small fraction of the vehicle following topical application. This can be accomplished by the use of appropriate volatile:nonvolatile solvent systems such that the large bulk of the vehicle is lost by evaporation immediately following application. The concentra-

tion of the drug in the remaining solvent can be increased many fold over the initial concentration depending on the nature and quantity of the nonvolatile solvent. Ideally, supersaturated systems can be attained having unusually high thermodynamic potentials. The formulations studied were dilute solutions of steroid in mixtures of isopropanol with either propylene glycol or isopropyl myristate. On application to the skin, the isopropanol rapidly evaporates leaving the steroid to concentrate into the nonvolatile component. The penetration through human skin from such systems has been determined using *in vitro* techniques.

EXPERIMENTAL

Preparation of Steroid Solutions—^{14}C-labeled fluocinolide[1] and fluocinolone acetonide[2] were prepared from solutions of high specific activity by diluting with nonradioactive steroid to about 2 μc./mg. and recrystallizing from ethanol and water. Steroid solutions were prepared by dissolving the dry powder in isopropanol, and aliquots were pipetted into separate vials. The solvent was removed by evaporation and the steroid in each vial was redissolved in one of the solvent mixtures of propylene glycol[3]/isopropanol[4] or isopropyl myristate[5]/isopropanol.[4] The specific activity of the reconstituted solutions was checked to ensure complete dissolution of the steroid.

Preparation of Skin Specimens—Whole thickness human abdominal skin obtained at autopsy was pressed onto a glass tile so that the epidermis was perfectly flat. The skin was frozen and stored in a freezer until required. Then it was removed from the tile and the epidermis allowed to soften. The surface remained flat, providing the subcutaneous layers were kept frozen, and the epidermis and most of the dermis were easily cut off as a sheet of tissue using a dermatome[6] set at 0.75 mm. The skin was wiped with a cotton tip soaked in methanol to remove any adhering fat and cut into small pieces of a size suitable for the skin cells.

Skin Cells—The skin cell (Fig. 1) was a modification of that used by Stoughton and Munro (9) and consisted of a lower glass chamber with a side arm to allow sampling of the receptor phase. A Teflon-coated stirring bar attached to a polyethylene sail provided efficient mxing. The Teflon disks holding the skin were clamped onto the flat ground glass surface at the top of the receptor chamber. The skin was sandwiched epidermis uppermost between the Teflon pieces, leaving exposed a central circular area 0.62 cm. diameter (0.25 in. diameter) through which the penetration was measured. A

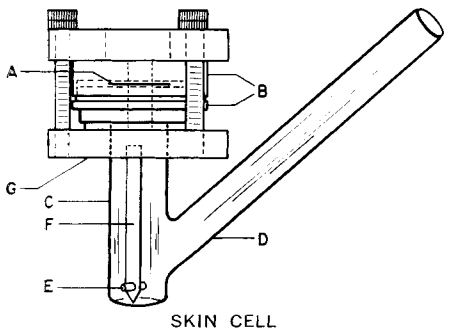

Figure 1—*The skin cell consists of a lower glass chamber with a side arm to allow sampling of the receptor phase. Key:* A, *skin specimen;* B, *Teflon pieces holding skin;* C, *receptor chamber;* D, *side arm;* E, *Teflon coated magnetic bar;* F, *polyethylene sail;* G, *clamp.*

[1] 6α,9α-Difluoro-16α-hydroxyprednisolone 16α,17α-acetonide 21-acetate, Syntex Research, Palo Alto, Calif.
[2] 6α,9α-Difluoro-16α-hydroxyprednisolone 16,17-acetonide, Syntex Research, Palo Alto, Calif.
[3] Propylene glycol USP, Union Carbide, New York, N. Y. 10017.
[4] Isopropanol AR grade, Mallinckrodt, St. Louis, Mo.
[5] Deltyl Extra, Givaudan Corp., Clifton, N. J. 07014.
[6] Padgett Electro Dermatome, Padgett Dermatome, Div. of Kansas City Assemblage Co., Kansas City, Mo. 64111.

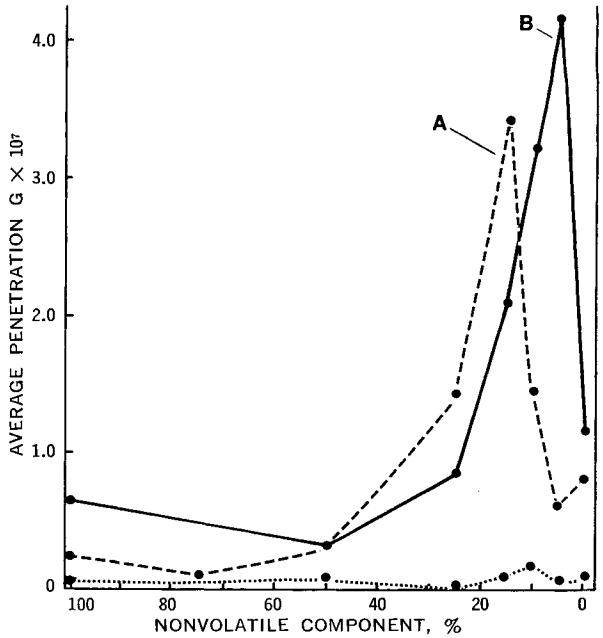

Figure 2—*Penetration of fluocinolide through human skin from solutions in isopropanol/propylene glycol after 16 hr. at 37°. Key:* ●···, *Solution occluded, 0.01%;* A, *initial fluocinolide concentration before evaporation of isopropanol = 0.05%;* B, *initial fluocinolide concentration before evaporation of isopropanol = 0.01%.*

solution containing 0.9% sodium chloride and 0.01% thimerosal was heated to expel dissolved gases, cooled, and 10 ml. was pipetted into each receptor chamber. The side arm was sealed with Parafilm and air bubbles were carefully removed from the dermal surface of the skin by tipping the cell. Each cell was then placed in a methyl methacrylate (Plexiglass) holder which fit over a magnetic stirrer in a 37° environmental chamber and sufficient time was allowed for temperature equilibration.

The radioactively labeled steroid solutions were pipetted onto the skin surface, usually in 0.05-ml. volumes, and the volatile isopropanol allowed to escape.

In some experiments silicone grease was smeared around the central well and the top of each cell was sealed with a glass cover slip to prevent evaporation of the cosolvent.

Radiochemical Assays—The penetration of the steroid after selected time intervals was followed by sampling the receptor solution and counting in a liquid scintillation counter (Nuclear-Chicago Unilux II). One-milliliter samples were removed *via* the side arm into 17 ml. of scintillation fluid consisting of 0.26 g. of POPOP[7](1,4-bis [2-(methyl-5-phenoxyazolyl)]benzene), 13 g. of PPO[7](2,5-diphenyloxazole), 208 g. of naphthalene,[8] 600 ml. of methanol,[9] 1 l. of toluene[10] and 1 l. of *p*-dioxane.[10]

The amount of quench due to the saline solution was determined by the channels ratio method, and the total amount of steroid penetration was then calculated.

Radiochromatograms of the solutions before and after penetration were developed to show that the steroids were not changed by their penetration through the skin.

RESULTS

Figures 2, 3, 4, and 5 show the average steroid penetration from each solution calculated from between 4–13 individual experiments. For simplicity, the standard errors are not shown in Figs. 2 and 3 but they have been calculated and they are of similar magnitude to those of Figs. 4 and 5.

Fluocinolide in Mixtures of Isopropanol and Propylene Glycol or Isopropyl Myristate—Figure 2 shows the penetration of fluocino-

[7] Arapahoe Chemicals, Division of Syntex Corp., Boulder, Colo.
[8] J. T. Baker Chemical Co., Phillipsburg, N. J.
[9] Mallinckrodt Chemical Works, St. Louis, Mo.
[10] Matheson, Coleman and Bell, Norwood, Ohio.

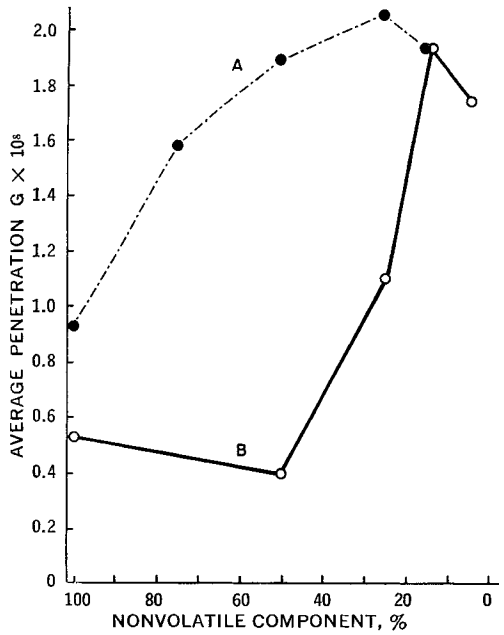

Figure 3—*Average total penetration of fluocinolide from 0.01 and 0.05% solutions in isopropyl myristate/isopropanol through the same skin specimens after 16 hr. at 37°. Key:* A, *initial concentration before evaporation of isopropanol = 0.05%;* B, *initial concentration before evaporation of isopropanol = 0.01%.*

lide through human skin after 16 hr. at 37° from various mixtures of propylene glycol and isopropanol. Very little penetration was achieved from the 0.01% steroid solutions containing up to 75% isopropanol, but increasing the amount of isopropanol beyond 75% results in an appreciable increase in penetration. The highest penetration was achieved from the 95% isopropanol/5% propylene glycol system. The graph also shows the small degree of penetration which occurred when evaporation of the isopropanol was prevented by occlusion.

Also included in Fig. 2 is the penetration from 0.05% fluocinolide solutions containing propylene glycol and isopropanol. Once again, a significant penetration was not achieved until the solvent system contained 75% volatile component. However, maximum penetration occurred from the 85% isopropanol/15% propylene glycol system and increasing the isopropanol content still further resulted in a decrease in penetration.

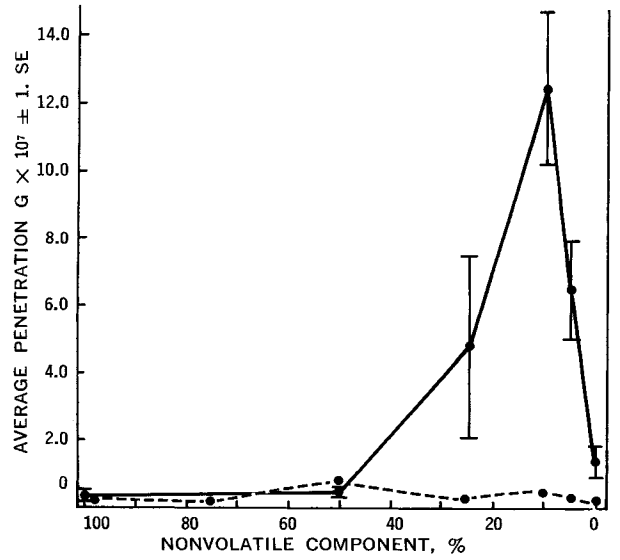

Figure 4—*Penetration of fluocinolone acetonide through human skin from 0.05 ml., 0.025% solution in isopropanol/propylene glycol after 16 hr. at 37°. Key:* ●—●, *not occluded;* ●---●, *occluded.*

As depicted in Fig. 3, the penetration of fluocinolide from solutions of isopropyl myristate and isopropanol was much lower than from the propylene glycol system, but even so, it showed a considerable enhancement as the proportion of volatile component was increased. In the 0.01% fluocinolide solution, a maximum in the degree of penetration was reached when 85% of the vehicle had evaporated, whereas with the 0.05% concentration, the maximum penetration occurred from the system containing 75% of volatile component.

Fluocinolone Acetonide in Mixtures of Isopropanol and Propylene Glycol or Isopropyl Myristate—Figure 4 shows the penetration of 0.025% fluocinolone acetonide from solutions of propylene glycol and isopropanol. Pure propylene glycol and solutions containing up to 50% isopropanol produced very poor penetration of the steroid. Increasing the isopropanol content to 75% increased the amount of fluocinolone acetonide transferred to about 4% of the total available for penetration. A further increase to a maximum transfer of about 10% of the membrane load was achieved by increasing the volatile component to 90% of the solvent system. The penetration from the 95% isopropanol/5% propylene glycol system decreased to about 5%.

As shown in Fig. 5, solutions of 0.025% fluocinolone acetonide in isopropyl myristate or in mixtures of isopropyl myristate with up to 75% isopropanol did not allow penetration through the skin of more than 1–2% of the total steroid concentration after 16 hr. However, the penetration was increased to about 5% of the total steroid concentration when the volatile cosolvent comprised 90–95% of the solvent system. When all the solvent was allowed to evaporate, penetration was very low.

Penetration was also very low from all the solutions when evaporation of the cosolvent was prevented.

DISCUSSION

It is evident that the enhancement of percutaneous penetration seen in these experiments is due to the increase in the solute concentration caused by the evaporation of the volatile component. These penetration effects cannot be due to the isopropanol exerting a defatting or carrier mechanism in the skin as the penetration is very low from the completely volatile solvent, and from all the occluded systems where evaporation was minimized and the solution remained on the skin surface throughout the experiment.

Figure 6 depicts the increase in concentration of fluocinolide in the nonvolatile propylene glycol relative to the concentration of a saturated solution as increasing amounts of the vehicle are allowed

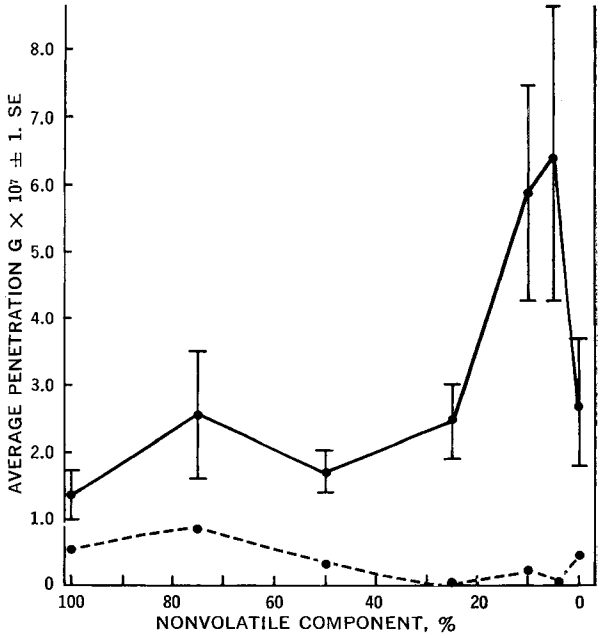

Figure 5—*Penetration of fluocinolone acetonide through human skin from 0.05 ml., 0.025% solution in isopropanol/isopropyl myristate after 16 hr. at 37°. Key:* ●—●, *not occluded;* ●---●, *occluded.*

to evaporate. This ratio gives an approximation to the thermodynamic activity of fluocinolide developed in the propylene glycol after the isopropanol has volatilized.

A saturated solution represented by a ratio of unity, should develop when about 92% of the vehicle has evaporated from the solution in which the initial concentration of fluocinolide was 0.01%. Similarly, the solutions containing 0.05% fluocinolide reach saturation when 60% of the solution has evaporated. Table I summarizes information for both steroids in either the propylene glycol or the isopropyl myristate systems.

Using this information on the development of more concentrated solutions, it is possible to interpret the penetration curves more fully.

For example, the penetration of fluocinolide from a 0.01% solution in propylene glycol (Fig. 2) is very low and the penetration does not increase significantly until about 75% of the vehicle has evaporated. At this point, the concentration profile (Fig. 6) shows a marked increase toward unity and the loss of additional small volumes from the vehicle causes large increases in the concentration of the steroid into the nonvolatile propylene glycol component. Corresponding closely to these increases are the large increases in penetration. When about 92% of the vehicle has evaporated, the remaining propylene glycol should be saturated with fluocinolide and higher penetration can be achieved only if some degree of supersaturation is attained. In fact, the maximum penetration does occur from the 95% isopropanol/5% propylene glycol solution.

The 0.05% fluocinolide solution in propylene glycol also shows a low degree of penetration and only a small increase in penetration is achieved until more than 50% of the vehicle has volatilized. Increasing the proportion of volatile component in the vehicle beyond 50% causes large increases in penetration up to a maximum at 85% volatile. This also relates to the concentration profile in Fig. 6. It is apparent that in this system the penetration from the saturated solution is less than maximal, and it seems likely that some degree of supersaturation has been achieved. Increasing the volatile component still further results in precipitation and a decrease in the degree of penetration.

The penetration of fluocinolide from 0.01% solutions in isopropyl myristate and isopropanol, depicted in Fig. 3, follows a similar pattern and relates closely to the concentration profile given in Table I. A rather different situation is found in the 0.05% steroid system since fluocinolide is much less soluble in isopropyl myristate than in propylene glycol. The saturation solubility is about 0.04% at 37° and the 100% isopropyl myristate system is actually a saturated solution with excess steroid in suspension. However, peak penetration does not occur from this suspension;

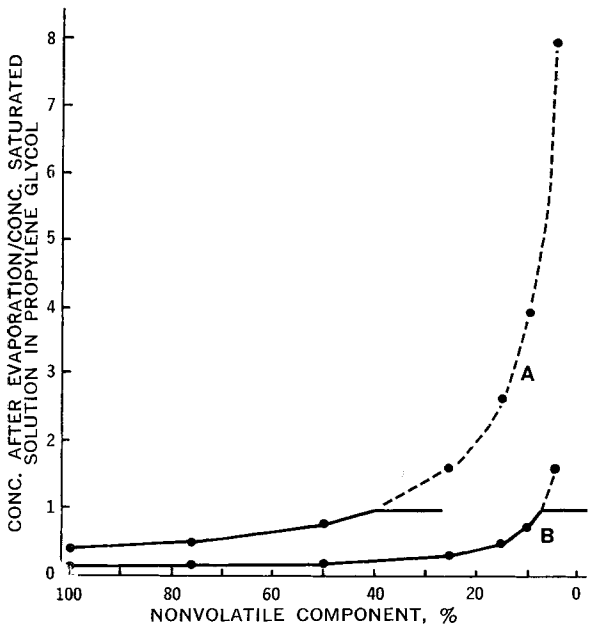

Figure 6—*Concentration profile of fluocinolide in propylene glycol at 37°. Key:* A, *initial fluocinolide concentration before evaporation of isopropanol = 0.05%;* B, *initial fluocinolide concentration before evaporation of isopropanol = 0.01%;* ——, *saturation.*

Table I—The Concentration Profiles of Fluocinolide and Fluocinolone Acetonide in Propylene Glycol or Isopropyl Myristate

	(Concn. of Steroid after Evaporation of Isopropanol)/(Concn. of Saturated Solution)		
	C_0 = 0.01%[a] Fluocinolide	C_0 = 0.05%[a] Fluocinolide	C_0 = 0.025%[a] Fluocinolone Acetonide
Propylene Glycol, Percent			
100	0.08	0.40	0.006
75	0.11	0.55	0.008
50	0.16	0.80	0.013
25	0.32	1.60	0.025
15	0.56	2.68	0.042
10	0.8	4.0	0.06
5	1.6	8.0	0.13
Isopropyl Myristate, Percent			
100	0.27	1.35	0.25
75	0.36	1.8	0.33
50	0.54	2.7	0.50
25	1.08	5.4	1.0
15	1.81	9.2	1.68
10	2.7	13.5	2.5
5	5.4	27.0	5.0

[a] C_0 = initial concentration.

it occurs from the solution initially containing 75% volatile solvent where, it is believed, an appreciable degree of supersaturation has been achieved.

The penetration curves of fluocinolone acetonide from 0.025% solutions in isopropanol and isopropyl myristate (Fig. 5) show similar features to those of fluocinolide. The system produces greater steroid penetration than it did with fluocinolide, but the same trend of increasing penetration to a maximum is observed as the thermodynamic activity of the steroid is increased by evaporation of isopropanol. The concentration profile (Table I) shows that the solution should become saturated when about 75% of the vehicle has evaporated, and since the penetration is increased beyond this point, it appears that some degree of supersaturation is developed in the system.

The penetration curves of fluocinolone acetonide from isopropanol and propylene glycol mixtures (Fig. 4) do not correspond as closely with the concentration profile as those of fluocinolide. It was found that the solubility of fluocinolone acetonide in propylene glycol was high, therefore, large increases in concentration could be achieved before excess steroid crystallized out of the solution.

Even the 20-fold increase in concentration created by volatilization of 95% of the vehicle should not theoretically saturate the remaining propylene glycol. Therefore, compared with the results for fluocinolide, it was expected that the penetration would continue to increase and not show a maximum within these solvent mixtures. However, the penetration does reach a maximum when about 90% of the vehicle has evaporated.

This may well be due to absorption of propylene glycol by the skin causing an additional concentrating effect on the steroid in the remaining vehicle. Possibly in this way, the 90% volatile system would become supersaturated, whereas, the 95% volatile system would cause precipitation and so reduce the penetration.

SUMMARY

An *in vitro* study was conducted to examine the percutaneous penetration of fluocinolide and fluocinolone acetonide from vehicles containing different proportions of isopropanol and either propylene glycol or isopropyl myristate. Penetration was enhanced to a maximum in all the systems as the volatile cosolvent isopropanol was allowed to evaporate. The increase in skin penetration is accounted for by the increase in the thermodynamic activity of the

steroid in the nonvolatile vehicle. The eventual decrease in penetration was the result of steroid precipitation from the supersaturated solution. In those cases where evaporation was prevented, the penetration from each solvent system remained at a low level.

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Model Catalysts Which Simulate Penicillinase IV: Steric and Electronic Effects in the Catalysis of Hydrolysis of Penicillins and Cephalothin by Aminoalkylcatechols

RENAAT D. KINGET* and MICHAEL A. SCHWARTZ†

Abstract □ Aminoalkylcatechols have been shown to catalyze the hydrolysis of penicillin at relatively rapid rates at neutral pH by a mechanism similar to that postulated for several hydrolytic enzymes. The present study was concerned with the effect on catalytic rates of varying the penicillin side chain and nucleus. Accordingly rates of hydrolysis of a number of penicillins and cephalothin were measured in the presence of several 3,6-bis(aminoalkyl)catechols where the amino group was varied in both size and basicity. Rates of alkaline hydrolysis were measured for comparison. There was little indication of steric hindrance of catalysis between penicillins with bulky side chains and catalysts with large amino groups. Electrostatic effects were much more prominent as exemplified by faster rates with carbenicillin (α-carboxybenzylpenicillin) than expected and changes in the pH-rate profile with ampicillin (α-aminobenzylpenicillin). With cephalothin the catalyst was virtually ineffective, probably because interaction between the carboxyl of a cephalosporin and a charged amine on the catalyst would leave the phenolate ion out of position for nucleophilic attack upon the β-lactam.

Keyphrases □ Aminoalkylcatechols—penicillinase simulation □ Penicillins, hydrolysis—aminoalkylcatechols, catalysts □ Cephalothin hydrolysis—aminoalkylcatechols, catalysts □ UV spectrophotometry—hydrolysis determination

Previous studies in this laboratory have shown that 3,6-bis(dimethylaminomethyl)catechol (CDM) (1) and other bis(aminoalkyl)catechols (2) are powerful catalysts of penicillin hydrolysis. Many of the characteristics of this catalysis resemble those observed with a number of hydrolytic enzymes. These include a maximum in the pH-rate profile (1), the presence of an acyl-catalyst intermediate (3), and some specificity in the structure of catalyst necessary for optimum activity (1). It has been shown that both the basicity of the amine and the susceptibility of the β-lactam to nucleophilic attack are factors influencing reaction rate.

The present study is concerned with the possible role of the penicillin side chain in the interaction with catalyst. A bulky side chain might sterically inhibit interaction of the penicillin with catalyst, whereas side chains containing certain groups might interact with the charged amino group and thus enhance catalytic rate. Therefore, the rates of hydrolysis of a number of penicillins were determined in presence of several 3,6-bis-(aminomethyl)catechols in which the basicity and size of the amino group were varied. Also studied was cephalothin, one of the cephalosporins, a group of β-lactam antibiotics which differ from the penicillins in the distance between the carboxylate ion and β-lactam carbonyl group.

EXPERIMENTAL

Substrates—Cephalothin and penicillin V,[1] nafcillin,[2] carbenicillin,[3] ancillin,[4] and other penicillins,[5] were all used. Two of the penicillins were prepared as follows:

Methylpenicillin—(0.1 mole) 6-Aminopenicillanic acid (6-APA) was dissolved in water containing 0.5 mole $NaHCO_3$ and 0.15 mole acetic anhydride added with stirring. After 90 min., the mixture was cooled, acidified to pH 2 with phosphoric acid, and extracted three

[1] Eli Lilly and Co.
[2] Wyeth Laboratories.
[3] Beecham Research Labs.
[4] Smith Kline & French Labs.
[5] Bristol Laboratories.