AN ABSTRACT OF THE THESIS OF

Xiumei Ruan for the degree of Master of Science in Pharmacy presented on April 10, 1992.

Title: Vehicle and Enhancer Effects on Penetration of Acyclovir through Chicken and Cockatiel skin In Vitro

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Abstract approved :

The penetration of acyclovir (ACV) through avian skin in vitro was studied in a Franz diffusion chamber, and the lateral apteria of chicken and cockatiel skin was excised and used in the study. ACV penetration was investigated by varying vehicles (Propylene Glycol [PG], Ethanol [ET], Isopropyl Myristate [IPM], O/w cream base, Ointment base), enhancers (Azone [1-dodecylazacycloheptan-2-one], 7FU [1-farnesyl-ℰ-caprolactam], 7GU [1-N-geranyl-ℰ-caprolactam]) and concentrations of acyclovir and enhancers. The topical ACV formulations were applied as suspensions, an ointment, and o/w cream. A HPLC with UV detection was employed to determine the amount of ACV penetration that crossed the avian skin membrane.

ACV penetration through chicken and cockatiel skin from ACV suspensions with solvent ET, PG, or IPM was significantly

increased by the addition of a penetration enhancer ($P<0.05$). The extent of penetrated acyclovir was definitively affected by the type of penetration enhancers which were involved, and Azone provided the greatest enhancement. Although, solvent effect on ACV penetration through chicken skin from a single vehicle was: IPM > ET > PG, the greatest observed improvement occurred in vehicle PG after the penetration enhancer was added into the delivery medium.

ACV penetration from a 5% ACV o/w cream was fivefold greater than from a 5% ACV ointment (Zovirax 5%) by using chicken skin model. In o/w cream study, the penetration of ACV through chicken and cockatiel skin was also improved by the addition of a penetration enhancer, and 7FU offered superior cutaneous absorption of ACV to Azone and 7GU. The effect of the penetration enhancer on o/w creams affected not only by the type of enhancer, but by the concentration of an enhancer added.

VEHICLE AND ENHANCER EFFECTS ON PENETRATION OF ACYCLOVIR THROUGH CHICKEN AND COCKATIEL SKIN *IN VITRO*

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I would like to dedicate this thesis to my parent for their never ending love and encouragement.

I would also like to express my sincere appreciation to my adviser, Dr. J.M. Christensen for his continual guidance, enthusiasm and supervision throughout my graduate study.

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CHAPTER I

IN VITRO PENETRATION OF ACYCLOVIR THROUGH CHICKEN SKIN MEMBRANES

INTRODUCTION

The purpose for this research is to explore a new method for drug administration to birds. Such a technique might have great value in improving veterinary therapy. In comparison of animal therapeutics, exotic birds is becoming a significant and still growing sector of the veterinary patient population (1). Avian medicine for exotic species remains, however, in its infancy. Within the last ten years, many avian diseases have been newly characterized, and many medications have seen their first assessment in avian therapies (2). To evolve a system of medical treatments, new therapeutic techniques must be developed to meet the needs of avian biology. Conventional routes of therapy used in mammalian species are associated with great risk and /or low efficacy. Intravenous injections are frequently followed by hemorrhage, because the accessible veins are friable, and vulnerable to trauma. Losses of blood even in these small quantities (on the order of one to several ml) are significant in birds because of their diminutive body sizes, therefore, blood volumes. Oral administration may have the hazard of accidental respiratory aspiration (3). In addition, it is likely to be a less desirable route of administration in the bird than in the mammal, because of their short length of intestines, which limit the time available for drug absorption (4). Transdermal absorption would by comparison offer greater

safety in the technique of drug administration (5). The kinetics of transdermal absorption might also offer advantages in therapeutic efficacy, since the absorption phase is prolonged over many hours. The high metabolic rates in birds translate into faster metabolism in clearance of xenobiotics, necessitating a higher dosing frequency than in mammals (6). High dosing frequencies are not only inconvenient for flock treatments, but they also engender a risk of imposing stress on the patient as a result of the frequent handling and restraint. Route of administration characterized by prolonged absorption, as with transdermal administration would offer important advantage in lower dosage frequencies thereby decreasing the distress of restraint and the incidence of treatment injury (5). Also, the convenience of combined with the low dosage frequency topical application could make this route of administration an efficient means for flock treatments.

Percutaneous absorption involves passive diffusion of substance through the skin. Stratum corneum in the skin layer is known as the main resistance (6). Generally, permeation by the transdermal route first involves partitioning into the stratum corneum. The rate of percutaneous absorption is proportional directly to the drug concentration (dissolved in its vehicle), partition coefficient, and diffusion coefficient, inversely to skin thickness (6). Transdermal absorption is expected to be successful in birds, more so than

in mammals, because birds have much thinner skins (presenting less of a barrier to penetration than in mammals) and small body sizes (providing a desirable body volume to surface ratio, which would allow the accumulation of therapeutically significant drug concentrations) (3).

Pacheco's Parrot Disease (PPD) is a highly contagious acute, and often fatal herpesviral infection which occurs in psittacine birds (7). Clinical signs of PPD include: sudden onset of lethargy, fluffing of the feathers, yellow urates, and regurgitation. Seizures often occur in terminal stages of the disease. Mortality rates can be as high as 100 percent in some species of psittacine birds. Birds in excellent body condition may be found dead without any prior clinic signs (8).

Acyclovir was chosen as a model compound for the evaluation of transdermal absorption, because it is used to treat the herpes virus of Pacheco's disease in Psittacine birds.

Acyclovir (ACV) was discovered in 1974 (9). Clinical trials of ACV began in 1977 and the first topical form of the drug was available to physicians in 1982 (9). ACV has been used as an antiviral compound due to its low host cell toxicity (10). The value of ACV has been documented in the treatment of severe herpes simplex virus (HSV) infections in immunosuppressed hosts and in primary genital HSV infection upon its administration either intravenously, orally or

topically (11, 12).

Acyclovir occurs as a white, crystalline powder and has solubilities of 1.3 mg/ml in water at 25°C and 0.2 mg/ml in alcohol. The drug has pK_a's of 2.27 and 9.25 (13, 14) so it is ionized at physiological pH's (Figure I.1) . Absorption of orally administered acyclovir is slow, variable and incomplete. Its oral bioavailability is approximately 15-30% (14). For topical use, acyclovir is commercially available as an ointment in a polyethylene glycol vehicle (14). Clinical failure has been reported for topical ACV treatment in patients (15, 16). The rate ACV penetrates the stratum corneum in these patients is minimal and may not reach infected cells in the lower layers of epidermis in adequate concentrations for therapy (15).

Several approaches have been used to improve the percutaneous absorption of acyclovir (17, 18, 19, 20, 21). Organic solvents {Ethanol(ET), Isopropanol(IPA), Propylene glycol(PG) Isopropyl myristate(IPM)} have been reported as vehicles to improve penetration of topical acyclovir (17). Cooper, et,al, demonstrated that the oleic acid and oleyl alcohol, when added to the polar solvent PG, greatly improved skin permeability to ACV (18) as did long-chain fatty acids and alcohols in conjunction with PG (19). Dimethyl Sulfoxide (DMSO) also accelerated the rate of skin penetration of ACV <u>in vitro</u> using guinea pig skin (15). A modified aqueous cream vehicle of 5% ACV (ACV-MAC) for the treatment of recurrent

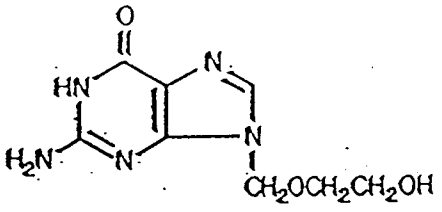


Figure I.1 Structure of acyclovir

Name: 9-[(2-Hydroxyethoxy)methyl]guanine

Formula: $C_8H_{11}N_5O_3$

Molecular weight: 225.21

Action: Antiviral.

Chemistry: White, crystalline powder; 1.3 mg/ml in water at 25^0C and 0.2 mg/ml in alcohol; Pk_a's 2.27 and 9.25; melting point 256^0C.

herpes labialis had superior cutaneous absorption relative to the commercial topical ACV formulation (13). Therefore, selecting a proper vehicle may enhance the penetration of acyclovir by favoring drug release or altering the physicochemical properties of skin membranes to alter drug penetration (22, 23).

Percutaneous penetration enhancers have been shown to improve the percutaneous absorption of ACV. Choi, et, al, demonstrated that the permeation of ACV increased systematically with increasing N-decylmethyl sulfoxide (NDMS) concentration. The action of NDMS was assessed by pretreating skin sections for various periods of time with the enhancer and then assessing permeability (19). Five choline esters, lauroylcholine, myristoylcholine, palmitoylcholine, stearoylcholine and oleoylcholine were evaluated as skin penetration enhancers of ACV by testing their effects on the penetration of ACV across hairless mouse skin <u>in vitro</u> and comparing the results to those obtained with oleic acid. The results showed that the transdermal delivery of ACV, tested in PG vehicle systems, could be significantly increased by adding a small amount of choline esters or oleic acid. Lauroylcholine and oleic acid were reported to act as synergists giving larger enhancement of ACV than when used separately (20). Compounds which contain an azacyclo ring with terpene and/or alkyl chain group, for example: 1-dodecylazacycloheptan-2-one(Azone), 1-geranylazacycloheptan-2-one

(7GU), 1-farnesylazacycloheptan-2-one(7FU) and 1-geranylaza-cyclopentan-2-one(5GU), have been reported as effective percutaneous penetration enhancers for ACV penetration through excised hairless mouse and rat skin (17). The mechanism of enhancer action for improving penetration of drugs might be reversible removal of the resistance barrier of the stratum corneum by modifying either the postulated lipid or polar route through the skin or both, which permits the unhindered access of a drug to the viable tissue and systemic circulation (21, 22, 24).

The objective of this study involves development of topical ACV formulations. The whole project was divided into pilot (optimizing ACV vehicle systems) and target (cockatiel) studies. Broiler hens, were chosen as an initial model to decrease cost for initial studies determining the optimum vehicle system and enhancers from numerous topical ACV formulations being tested before applying the results to the more valuable cockatiels. In the pilot study, effects of vehicles and the percutaneous absorption enhancers on the penetration of acyclovir from topical formulations were determined in the chicken lateral apteria skin model <u>in vitro</u>.

MATERIALS AND METHODS

Materials and chemicals

Acyclovir(a gift) and Zovirax ointment 5% were obtained from Burroughs Wellcome Co., Research Triangle Park, NC; Azone(1-dodecylazacycloheptan-2-one) was donated by Whitby Res. Inc., Richmond VA; 7FU(1-farnesyl-ℰ-caprolactam) and 7GU (1-N-geranyl-ℰ-caprolactam) as gifts were provided by Kuraray Co., LTD. Japan; Saline was obtained form Abbott Lab. North chicago, IL; Ethanol was obtained from Georgia Pacific Inc., Downmohan, WA; Propylene glycol (PG) and materials for o/w cream base including Sesame oil, Glycerol monostearate, Tween 80, Span80, Trithenolmine and Carbopol 940 were obtained from Merck & Co.,Inc. Rahway, NJ; Hypoxanthine and Isopropyl myristate were obtained from Sigma Chemical Co., St. Louis, MO); Broiler hens were raised and provided by Department of Poultry Science, Oregon State University, Corvallis, OR.

Preparation of Skin Samples

Broiler hens weighing 1.2-1.9 Kg (5-7 weeks) were used in this study. Five chickens were sacrificed each day by restraining them upside down in an inverted cone and slicing through the neck to sever the jugular and carotidal vessels. After plucking down the feathers, and removal of adherent fat,

fascia, and skeletal muscle from the undersurface, ten pieces of 6-7 cm^2 lateral apteria skin samples were obtained and placed on a gauze sponge. In order to keep skin fresh, excised samples were laid on gauze sponge moistened with physiological saline and sealed in a zip-locked plastic bag (Baggies® sandwich bags). The bags were then placed on ice. The skin was again cleaned in saline before being mounted in the Franz diffusion cells.

Experimental Design

To optimize ACV penetration through chicken skin by varying vehicles, enhancers, concentrations of acyclovir and a penetration enhancer, experiments were performed among forty-one topical ACV applications in three dosage forms (suspension, o/w cream and a commercial ointment [Zovirax Ointment 5%]) with three solvents making suspensions (Ethanol [ET], Propylene Glycol [PG], and Isopropyl Myristate [IPM]), an emulsion base and ointment (o/w cream base (21) and Polyethylene Glycol [PEG] base) and three penetration enhancers {Azone, 7FU (1-farnesyl-ℰ-caprolactam, and 7GU (1-N-geranyl-ℰ-caprolactam)}. It was a completely random design with each treatment duplicated or repeated, and triplicate replications for each treatment. The formulae and experimental design are summarized in Table I.1.

In Vitro Percutaneous Penetration Experiment

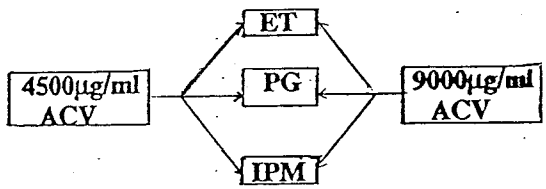
Percutaneous penetration of ACV was studied in vitro in an upright Franz diffusion cell. Samples were mounted in donor cells with an available diffusion area of 3.14 cm^2. The receptor compartment of each cell was filled with 14 ml of saline and stirred with a magnetic stirrer. Test formulations, except the commercial ointment (Zovirax Ointment 5%), were prepared by (I) suspending acyclovir in solvent {ET, PG or IPM) to an ACV dose of 4500 μg/ml (20 mM) or 9000 μg/ml (40mM)} with or without 0.2M of penetration enhancer, either Azone, 7FU or 7GU; (II) mixing acyclovir and an o/w cream base to form 5% ACV cream, and then adding 0.0%, 1.0% or 2.0% enhancer, either Azone, 7FU or 7GU. An ACV suspension (2.0ml) or ACV o/w cream 5% (1.0g) was applied to the donor compartment, which was then sealed at the top with parafilm to prevent evaporation. The diffusion cell was maintained at 37^0C (±0.5°C) by circulating water around the cell from a heated water bath. Selected sampling times were 0, 1, 2, 4, 6, 8, 12 and 20 hours after application of the topical formulation to the skin membrane. At the above time intervals, 0.8 ml of the receptor medium was withdrawn as a sample and this volume was replaced with fresh medium.

Analytical Assay for Penetration of Acyclovir

The amount of acyclovir penetrated was determined by HPLC (M-15 Waters Associate Inc., MA). The mobile phase consisted of a mixture of methanol and distilled water (10:90), the column was SELECTISOL 10-cm C18 (5-micron, Phenomenex Inc., Torrance, CA). Detection was performed by UV (Model 440 Waters Associate Inc., MA) absorbance at 254 nm with a flow rate of 0.9 ml/min and a pressure of 2100 PSI, and hypoxanthine (3.0 μg/ml) was used as the internal standard. Samples obtained from receptor cells were analyzed after filtration with a 5-μm filter needle, and then centrifuged 8 min at a speed of 9000 rpm which yields 760 g force (Centrifuge 5415C, Brinkmann Instruments, Inc. Germany).

Table I.1 Experimental design for diffusion study using chicken skin model

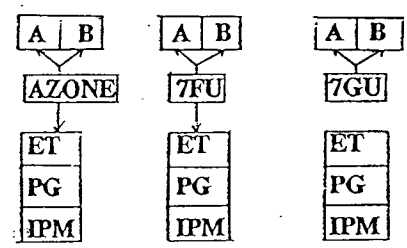
1. EFFECTS OF VEHICLES



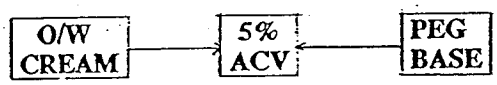
2. EFFECTS OF ENHANCERS

A = 4500μg/ml ACV;

B = 9000μg/ml ACV



* = 1.0%; ★ = 2.0%



DOSAGE FORM	ACV DOSE	FORMULA VEHICLE	ENHANCER		No. OF TESTS
Suspension	4500μg/ml or 9000μg/ml	ET, PG, IPM	with 0.2M or without	Azone, 7FU, 7GU	21 12
O/w cream	5%(w/w)		with 1.0% or 2.0% or without	Azone, 7FU, 7GU	7
Ointment	5%(w/w)	PEG	without		1

RESULTS AND DISCUSSION

Vehicle Effects on Penetration of Acyclovir

(I) Suspension The cumulative penetration of ACV suspended in the solvents Ethanol, Propylene glycol or Isopropyl myristate through chicken lateral atperia skin is presented in Table I.2 and I.3. The variation of cumulative ACV penetration among the three solvents at twenty hours was significantly different ($P < 0.05$). The solvent IPM allowed the maximum penetration at doses 4500 μg/ml (20mM) and 9000 μg/ml (40mM) yielding a cumulative amount penetrating through of 41.93 μg and 45.367 μg, respectively. The delivery of ACV varied among solvents and ACV penetration through chicken skin ranked as follows: IPM > ET > PG (Figure I.2, I.3). The vehicle IPM {δ=8.54 $(cal/cm^3)^{1/2}$} is shown as the most hydrophobic compound among the three selected solvents (Table I.4), thus, the lipophilic vehicle (δ_{IPM}=8.54) makes ACV penetrate through the skin barrier easier than the vehicle (δ_{PG}=14.8) with hydrophilicity does. The difference in penetration of acyclovir among these vehicles indicated the importance of selecting a proper vehicle. According to diffusion equation: $M = P\ C_d\ t$ (6), the extent of drug penetration (M) through skin membrane is directly proportional to the concentration of that drug (C_d) dissolved in a vehicle, the skin permeability ($P = DK/h_{sc}$), and the diffusion time (t). Skin permeability (P) is a function

of diffusion coefficient (D), partition coefficient (K), and the thickness of stratum corneum (h_{sc}) in the skin layer. Therefore, the higher C_d and P, the smaller h_{sc}, the greater amount of drug penetrates (6, 24). The vehicle effect can influence either a direct action on skin permeability (in improving D or K, or both), or altering drug solubility (C_d) (17, 23, 25).

The variation of ACV penetration observed in different vehicles without enhancer is smaller than with penetration enhancers present (Tables I.5-I.11). The extent of enhancement of ACV penetration in different vehicles was shown to change significantly with or without an enhancer present ($P < 0.05$). With the addition of a penetration enhancer, the order for ACV penetration changed for the three organic solvents listed: PG > ET > IPM instead of IPM > ET > PG in a single vehicle. Since the greatest penetration was observed with solvent PG and enhancer Azone (Figures I.5-I.10), the combination of a hydrophilic solvent $\{\delta_{PG}=14.8(cal/cm^3)^{1/2}\}$ with a hydrophobic enhancer $\{\delta_{Azone}=9.07 (cal/cm^3)^{1/2}\}$ (Table I.4) is the desired combinstion for a suspension vehicle. Therefore, vehicles can also affect the action of the penetration enhancer, which added into that vehicle, on the penetrant in the different extent.

(II) <u>O/w Cream and Ointment</u> The penetration of ACV from o/w cream 5% and Ointment (Zovirax) 5% is shown in Table I.5.

Acyclovir released sooner from the o/w emulsion occurred and to a greater extent than from the commercial ointment during the 20-hour study period. The total ACV penetration (23.86 μg) was fivefold greater through chicken skin using o/w cream as a vehicle than with an ACV ointment (Figure I.4). There was a greater than 12-hour lag time before acyclovir began releasing from 5% ACV formulated in a Polyethylene glycol (PEG) base. The clinical failures documented (11, 14) could be in part due to poor penetration through skin of ACV in adequate amounts for therapy using the commercial 5% ACV ointment [Zovirax Ointment 5%]. O/w cream base (26) is formulated using IPM, PG, ET, and surfactants Span80, Tween80 in the vehicle. These may play a role as vehicles or enhancers or both, thereby promoting ACV release from the o/w cream (23).

Enhancer Effect on Penetration of Acyclovir

(I) Suspension Percutaneous penetration of acyclovir was significantly ($P<0.05$) improved by addition of the penetration enhancer. Tables I.6-I.11 summarize topical ACV penetration with or without enhancers. The slope of the ACV penetration profile positively shifts when the suspension was prepared with an enhancer of either Azone, 7FU or 7GU (Figure I.5-I.10). The effect of penetration enhancers increases with decreasing lipophilicity of the vehicle. The greatest

enhancement in a suspension appeared when acyclovir was mixed with Azone and PG. A promising amount of ACV penetration was obtained from the binary vehicle with PG as the solvent and enhancer Azone (Figure I.5-I.10). The maximum cumulative amount penetrating (88.40 μg and 102.15 μg ACV) appeared with PG and Azone at an ACV dose of 4500 μg/ml (20mM) and 9000 μg/ml (40mM), which was increased by over 85% of the total ACV penetration compared to PG vehicle itself. The order of enhancer effects on ACV released from the solvents among the three selected enhancers was: Azone > 7FU > 7GU (Figure I.13, I.14). Extent of enhancement in ACV penetration was definitely affected by both the type of enhancers and vehicles involved. The challenge is to select the optimal combination. For acyclovir, the proper vehicle can be considered to combine hydrophilic vehicle with lipophilic penetration enhancer (17). The reason for the improvement was due to the drug being applied in a binary vehicle system (organic solvent and penetration enhancer), or use of the combination of the percutaneous absorption promoter (Azone, 7FU, 7GU) and the vehicle ET, PG and IPM, which in either instance may alter the physicochemical properties of the skin barrier to increase the permeability coefficient, or match acyclovir properties to improve ACV solubility, or both (6, 23, 24).

(II) <u>O/w Cream</u> Percutaneous penetration enhancer improved penetration of ACV over the o/w emulsion formulation without

a penetration enhancer (Table I.12). Comparing results from 5% ACV cream with or without the penetration enhancer, percutaneous absorption of ACV increased by 60.93%, 68.52% and 53.84% with the addition of 2% of the penetration enhancer Azone, 7FU or 7GU to the formulation, respectively (Figure I.11, I.12). 7FU was the most effective enhancer for the o/w cream formulations (Figure I.13, I.14). The extent of ACV penetration was affected not only by the type of enhancer, but the concentration of the enhancer added. The cumulative penetration of ACV from a 5% cream with a 2% enhancer increased 1.3 times over that when 1% enhancer was added (Figure I.11, I.12). Thus, increasing the concentration of the percutaneous absorption enhancer can improve ACV penetration ($P<0.05$).

Dose Effect on Penetration of Acyclovir

No statistical variation ($P>0.05$) in the rate of ACV penetration through chicken skin were observed after doubling ACV doses in topical ACV suspensions, although, the cumulative ACV penetration from ACV suspensions increased with dose in the presence of an enhancer (Figure I.5-I.10). For example, ACV dose in a ACV/ET suspension with 0.2M Azone increased from 4500 μg/ml (20mM) to 9000 μg/ml (40mM), the ACV penetration at 20-hour also increased from 50.27 μg to 75.12 μg/ml. Wester examined the effects of concentration on percutaneous

absorption of nitroglycerin. Topical nitroglycerin dose (C_d=10 mg/cm^2) increased 10 times with the amount of material becoming systemically available also increasing to 10 times (23). Since acyclovir was prepared in a suspension, the solubility of ACV may be kept constant at two ACV doses level in the vehicle (single or binary), increasing amount of applied drugs in suspensions does not alter the concentration of acyclovir in that vehicle, and diffusion rate should be constant, thus, dose effect did not affect ACV penetration at $P<0.05$ experiment level.

Table I.2 Cumulative penetration of acyclovir through chicken skin from 4500 μg/ml ACV suspensions (n=6)

TIME (HR)	ET (μg)	PG (μg)	IPM (μg)
1	0.000	0.000	0.000
2	0.000	0.000	0.000
4	4.025±0.804	2.625±0.330	3.675±0.620
6	7.310±0.881	3.885±0.952	8.295±1.553
8	9.800±0.524	6.265±1.450	10.22±3.188
12	12.50±0.745	8.680±1.450	20.21±2.463
20	25.87±2.477	13.26±1.429	41.93±3.704

Table I.3 Cumulative penetration of acyclovir through chicken skin from 9000 μg/ml ACV suspensions (n=6)

TIME (HR)	ET (μg)	PG (μg)	IPM (μg)
1	0.000	0.000	0.000
2	0.000	0.000	0.000
4	3.920±0.581	3.896±0.837	4.736±0.732
6	5.572±0.819	5.740±0.501	7.980±0.806
8	8.876±0.843	8.144±1.152	11.25±0.701
12	12.82±0.608	10.64±1.155	23.77±1.322
20	26.12±2.348	14.69±1.217	45.36±3.356

Table I.4 Solubility parameters of vehicles and enhancers[17]

Compound	Solubility parameter (δ)* $(cal/cm^3)^{1/2}$
Vehicle	
Isopropyl myristate	8.54
Ethanol	12.6
Propylene glycol	14.8
Enhancer	
Azone	9.07
7FU	9.10
7GU	9.21

*: δ, solubility parameter, express the cohesion between like molecules using as an index of compound polarity. A large δ means high hydrophilicity of the compound.

Table I.5 Cumulative penetration of acyclovir (μg) through chicken skin from Zovirax 5% and o/w cream 5%

TIME (HR)	ZOVIRAX (n=6)	CREAM (n=6)
1	0.000	0.000
2	0.000	0.000
4	0.000	3.570±0.580
6	0.000	6.534±0.994
8	0.000	8.074±1.686
12	0.000	10.92±1.492
20	4.470±1.502	23.29±4.178

Table I.6 Cumulative penetration of acyclovir (μg) through chicken skin from 4500 μg/ml ACV/ET suspensions with 0.2M enhancers

TIME (HR)	CONTROL (n=5)	+AZONE (n=6)	+7FU (n=6)	+7GU (n=6)
1	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000
4	4.025±0.804	5.600±0.899	5.506±0.770	5.012±0.410
6	7.315±0.881	9.338±0.875	8.960±0.869	8.166±0.970
8	9.800±0.524	12.67±0.423	12.09±0.630	11.74±0.829
12	12.50±0.745	21.08±1.169	18.86±0.784	15.42±0.543
20	25.87±2.447	50.27±1.667	42.40±3.689	35.16±2.548

Table I.7 Cumulative penetration of acyclovir (μg) through chicken skin from 9000 μg/ml ACV/ET suspensions with 0.2M enhancers

TIME (HR)	CONTROL (n=6)	+AZONE (n=6)	+7FU (n=6)	+7GU (n=6)
1	0.000	0.000	0.000	0.000
2	0.000	4.434±0.361	4.574±0.648	4.434±.0657
4	3.920±0.581	8.600±0.539	7.560±1.559	6.930±1.084
6	5.572±0.819	13.17±0.770	11.06±1.596	9.194±0.678
8	8.876±0.843	23.91±0.261	18.74±1.162	12.61±0.449
12	12.82±0.608	39.70±1.504	29.25±3.417	20.34±2.150
20	26.12±2.348	75.12±4.376	65.04±3.888	46.01±2.443

Table I.8 Cumulative penetration of acyclovir (μg) through chicken skin from 4500 μg/ml ACV/PG suspensions with 0.2M enhancers

TIME (HR)	CONTROL (n=6)	+AZONE (n=6)	+7FU (n=6)	+7GU (n=6)
1	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000
4	2.625±.330	8.190±1.886	5.624±0.610	5.628±0.458
6	3.885±0.925	13.09±2.089	10.50±1.184	8.428±1.007
8	6.265±1.450	27.65±5.702	18.99±2.979	12.32±0.995
12	8.680±1.450	49.19±6.289	29.00±6.958	26.32±2.295
20	13.26±1.429	88.40±7.753	62.47±9.338	51.16±4.763

Table I.9 Cumulative penetration of acyclovir (μg) through chicken skin from 9000 μg/ml ACV/PG suspensions with 0.2M enhancers

TIME (HR)	CONTROL (n=6)	+AZONE (n=6)	+7FU (n=6)	+7GU (n=6)
1	0.000	0.000	0.000	0.000
2	0.000	7.070±1.005	4.876±0.823	5.904±1.135
4	3.896±0.837	10.73±1.812	8.190±1.460	10.31±1.929
6	5.740±0.501	17.43±4.148	16.06±1.474	17.64±6.261
8	8.144±1.152	35.53±8.120	29.95±1.835	25.20±8.093
12	10.64±1.155	62.37±9.551	52.03±5.106	45.66±5.489
20	14.49±1.217	102.2±6.478	82.40±9.190	69.30±2.471

Table I.10 Cumulative penetration of acyclovir (μg) through chicken skin from 4500 μg/ml ACV/IPM suspensions with 0.2M enhancers

TIME (HR)	CONTROL (n=6)	+AZONE (n=6)	+7FU (n=6)	+7GU (n=6)
1	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000
4	3.675±0.620	5.600±0.938	5.012±0.528	4.788±0.400
6	8.295±1.553	10.27±1.232	8.148±1.781	7.604±1.194
8	10.22±3.188	19.09±0.973	13.98±2.926	12.50±2.061
12	20.21±2.363	28.09±4.662	25.13±5.659	20.93±1.109
20	41.93±3.704	58.24±4.819	49.64±4.767	43.06±5.585

Table I.11 Cumulative penetration of acyclovir (μg) through chciken skin from 9000 μg/ml ACV/IPM suspensions with 0.2M enhancers

TIME (HR)	CONTROL (n=6)	+AZONE (n=6)	+7FU (n=6)	+7GU (n=6)
1	0.000	0.000	0.000	0.000
2	0.000	5.040±0.307	5.064±0.671	4.516±0.623
4	4.376±0.732	8.354±0.421	7.420±0.746	7.884±0.615
6	7.980±0.806	12.11±0.834	10.41±0.564	12.24±0.906
8	11.25±0.701	20.01±1.617	14.40±1.064	18.24±4.598
12	23.77±1.322	35.45±2.782	27.50±2.706	23.87±6.405
20	45.36±3.356	78.70±7.095	69.23±2.535	56.93±5.739

Table I.12 Cumulative penetration of acyclovir (μg) through chicken skin from o/w cream with or without enhancers

TIME (HR)	CONTROL (n=6)	+1%AZONE (n=6)	+1%7FU (n=6)	+1%7GU (n=6)
1	0.000	0.000	0.000	0.000
2	0.000	2.975±0.619	3.735±1.149	2.824±0.410
4	3.570±0.580	5.548±0.839	6.779±1.140	4.900±0.895
6	6.534±0.994	10.42±1.680	10.78±1.992	6.744±1.119
8	8.074±1.686	17.36±5.370	18.04±4.857	11.26±1.016
12	10.92±1.492	26.85±7.524	34.68±3.941	14.16±1.765
20	23.29±4.178	43.45±7.556	55.56±3.847	28.57±3.479
		+2%AZONE (n=6)	+2%7FU (n=6)	+2%7GU (n=6)
1		0.000	0.000	0.000
2		3.836±0.623	4.648±1.220	3.804±0.888
4		7.280±1.459	7.728±1.336	5.834±2.293
6		12.43±1.397	12.63±0.659	9.264±2.789
8		22.18±6.423	23.99±2.724	13.77±5.335
12		33.77±9.897	39.76±9.316	24.24±8.967
20		59.60±5.408	72.89±9.068	50.44±8.886

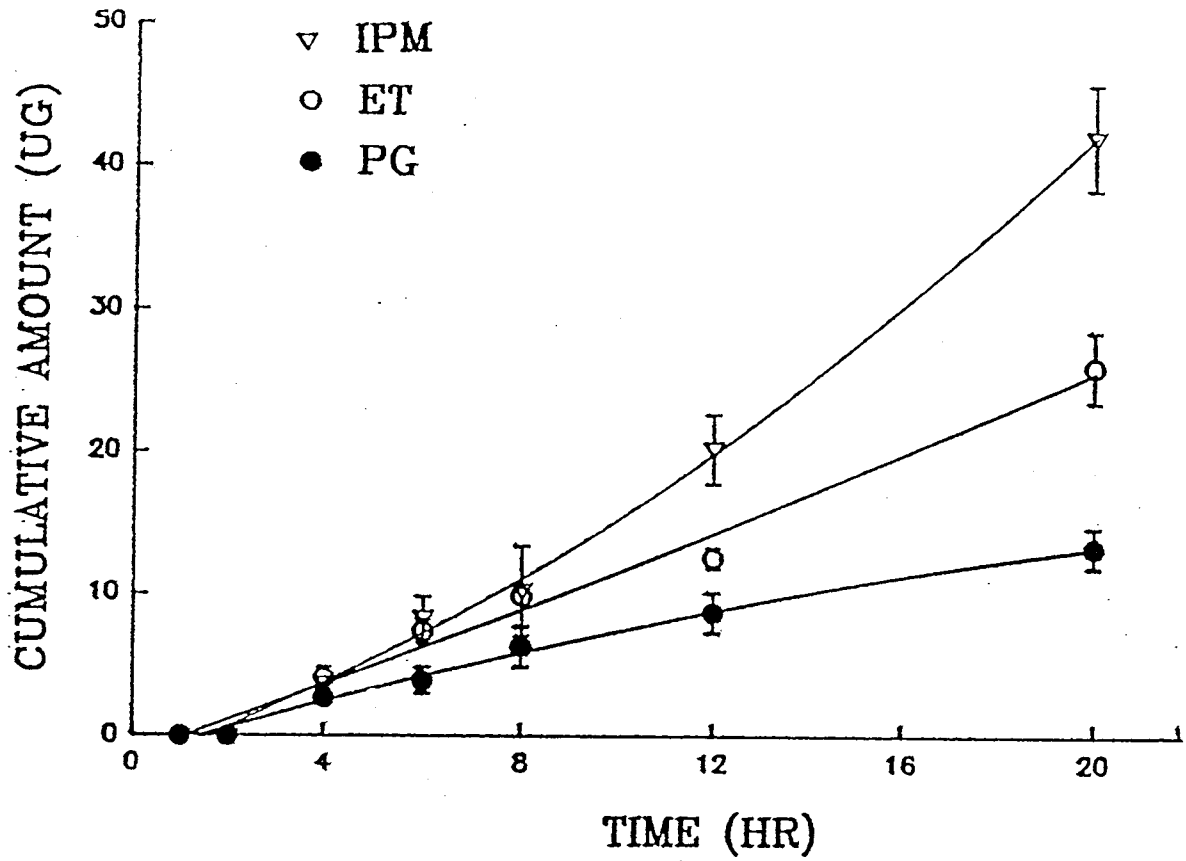


Figure I.2 Effect of vehicles on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 4500 μg/ml ACV suspensions with three organic vehicles in 20-hour diffusion study in vitro. Open circle, vehicle Ethanol; filled circle, vehicle Propylene Glycol; open triangle, vehicle Isopropyl Myristate. (n=5)

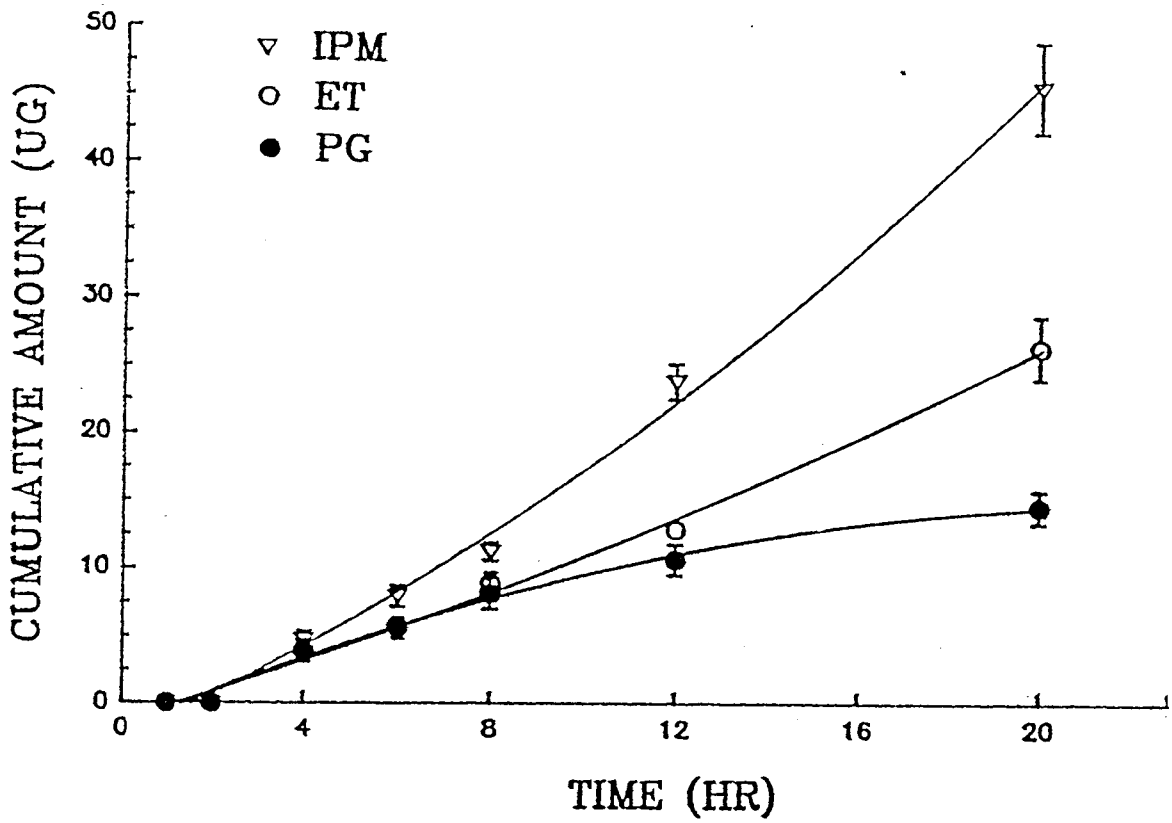


Figure I.3 Effect of vehicle on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 9000 μg/ml ACV suspensions with three organic vehicles in 20-hour diffusion study in vitro. Open circle, vehicle Ethanol; filled circle, vehicle Propylene Glycol; open triangle, vehicle Isopropyl Myristate. (n=6)

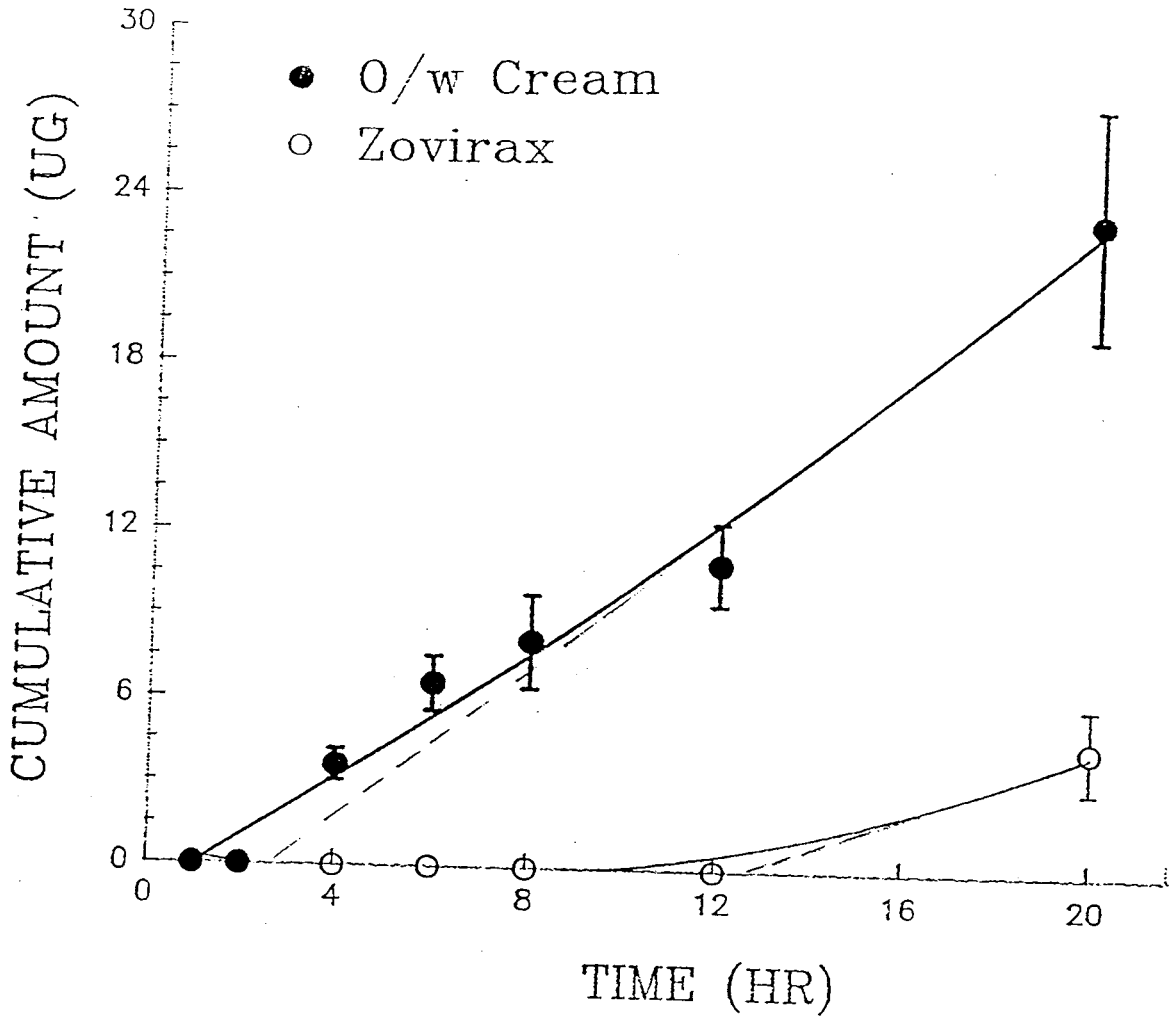


Figure I.4 Effect of vehicle on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from a 5% ACV ointment (Zovirax) and a 5% ACV o/w cream in 20-hour diffusion study in vitro. Open circle, vehicle Polyethylene Glycol; filled circle, vehicle o/w cream base. (n=6)

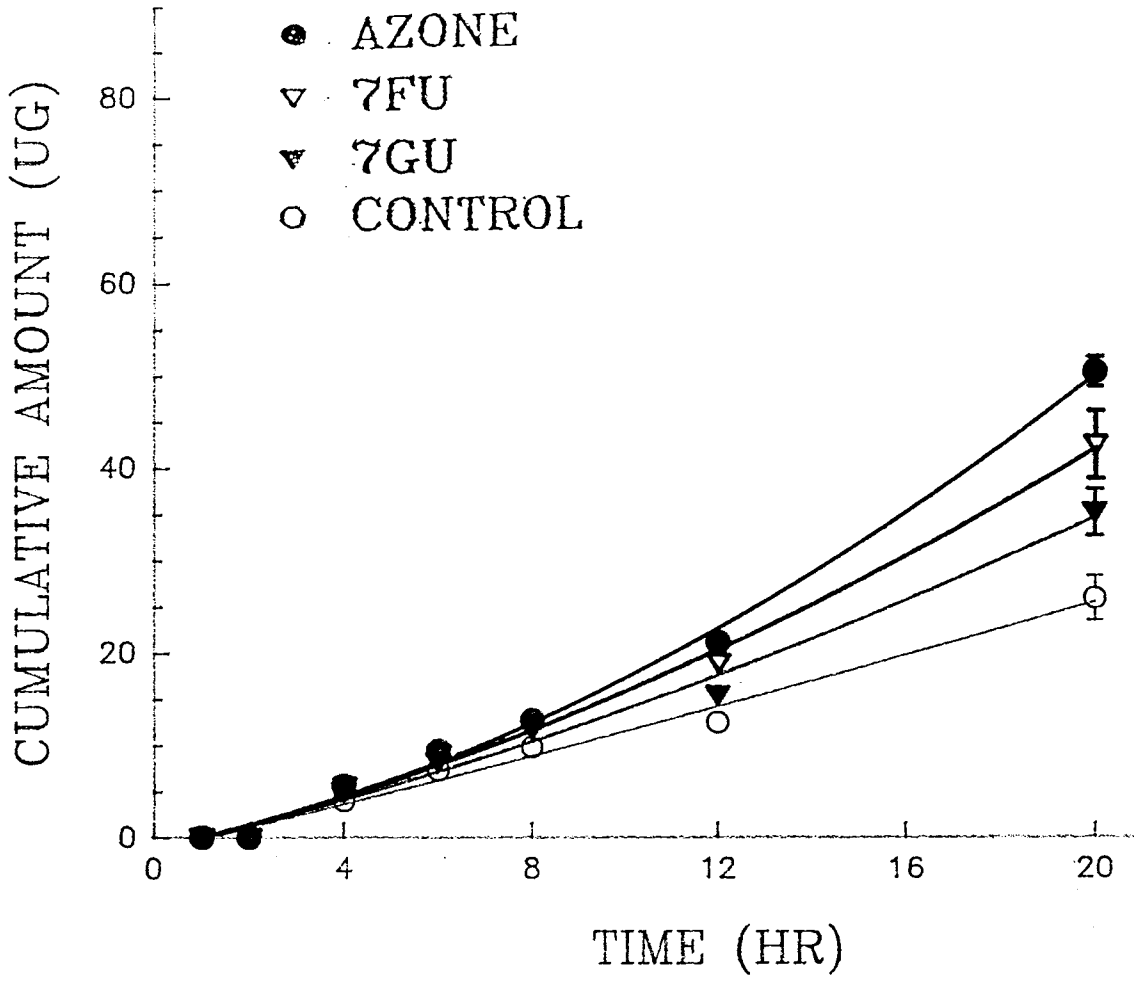


Figure I.5 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 4500 μg/ml ACV suspensions with vehicle Ethanol in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; filled triangle, with 7GU. (n=6)

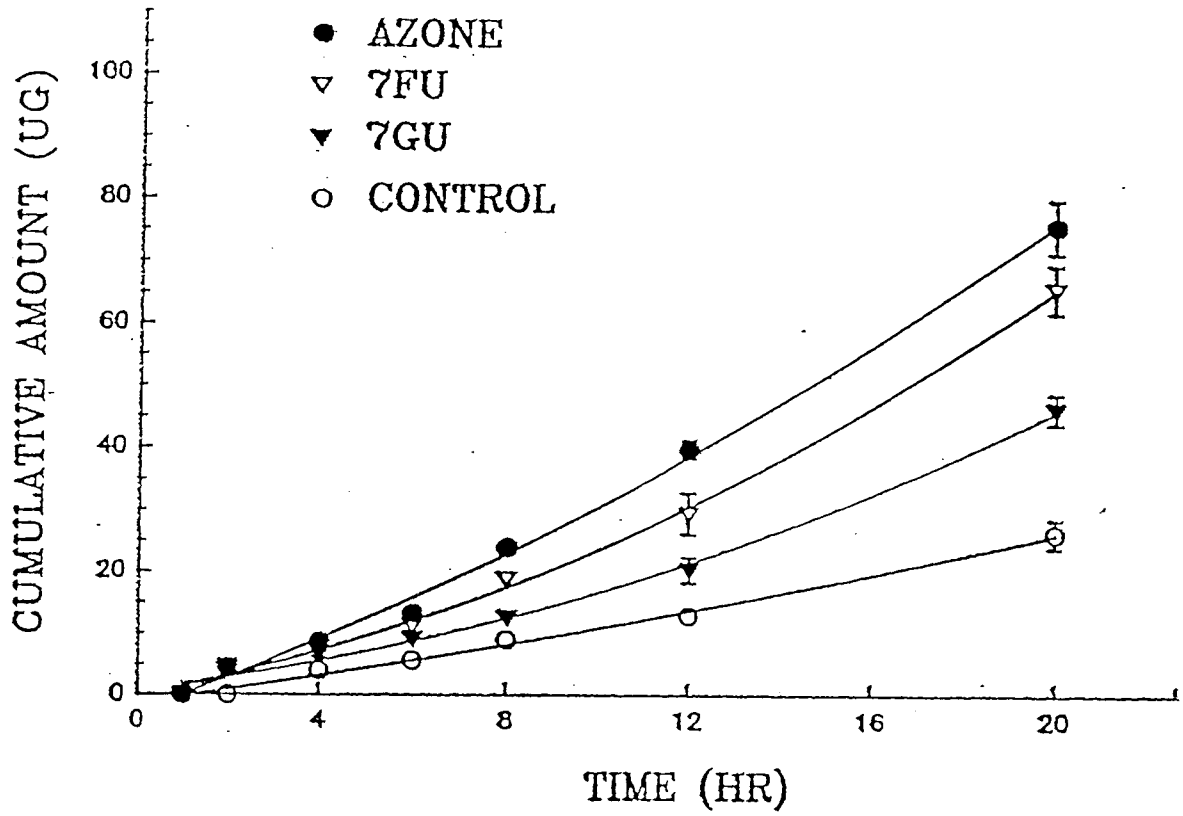


Figure I.6 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 9000 μg/ml ACV suspensions with vehicle Ethanol in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; filled triangle, with 7GU. (n=6)

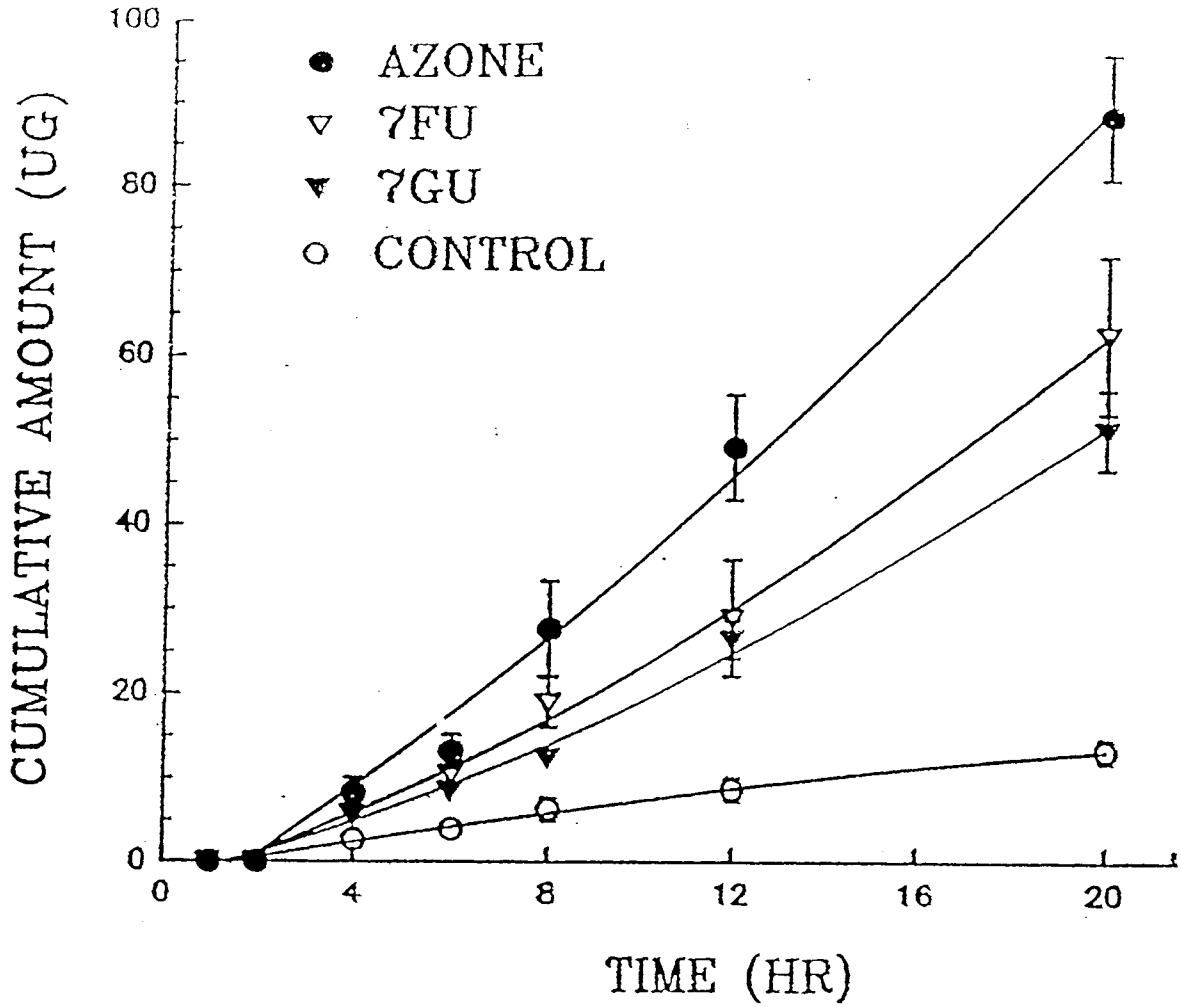


Figure I.7 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 4500 μg/ml ACV suspensions with vehicle Propylene Glycol in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; filled triangle, with 7GU. (n=6)

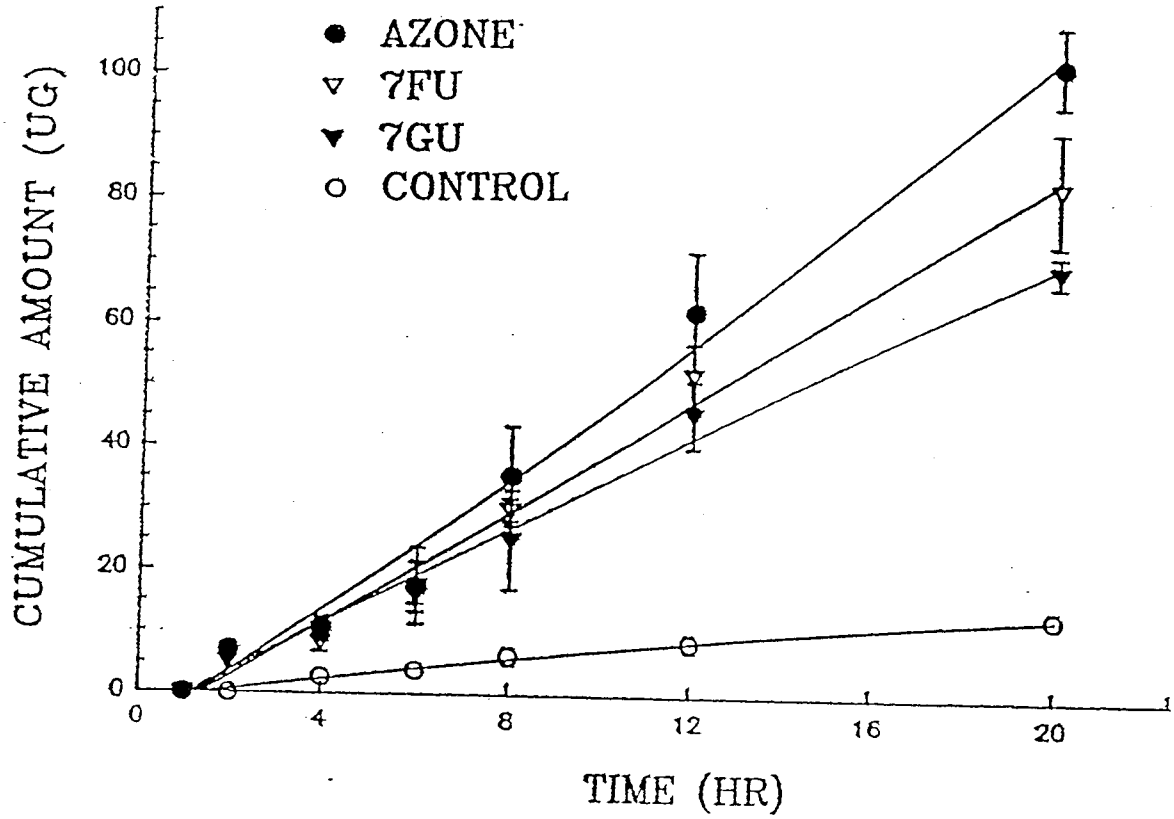


Figure I.8 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 9000 μg/ml ACV suspensions with vehicle Propylene Glycol in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; filled triangle, with 7GU. (n=6)

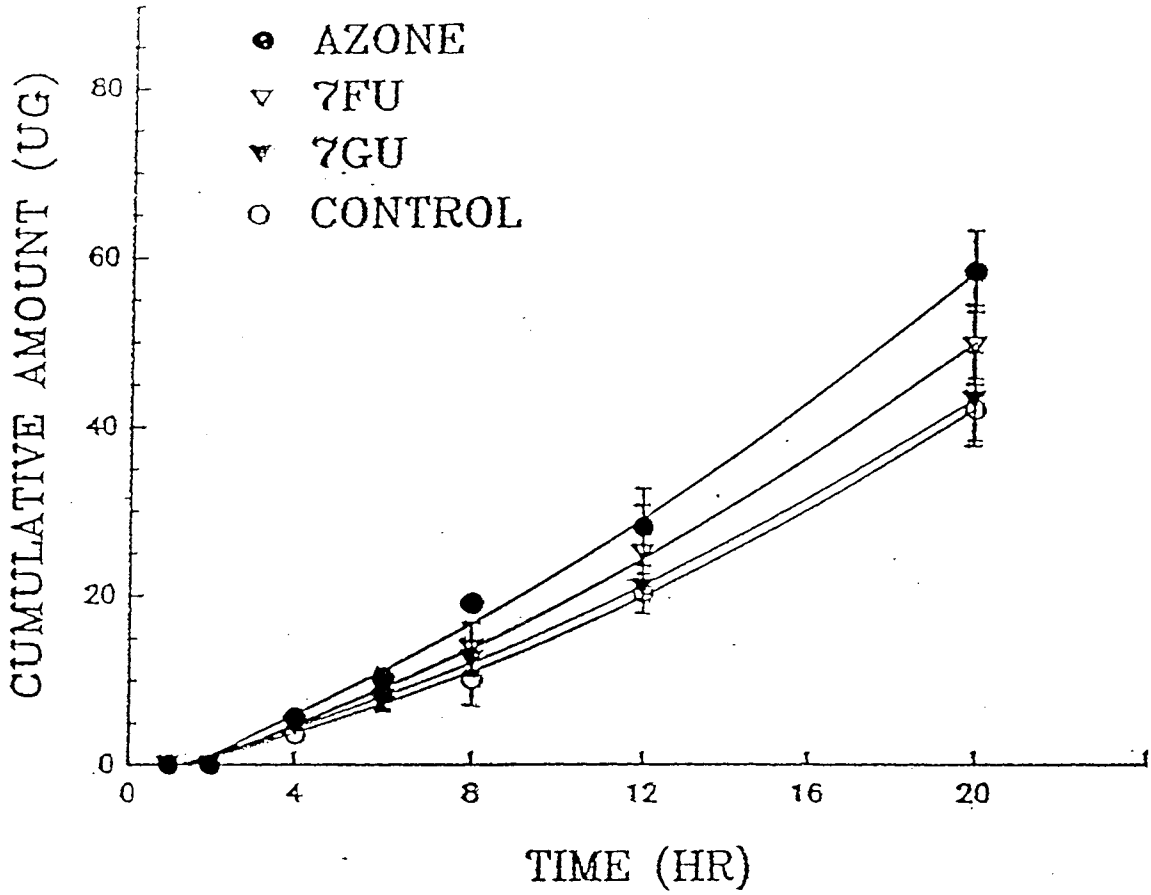


Figure I.9 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 4500 μg/ml ACV suspensions with vehicle Isoproyl Myristate in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; filled triangle, with 7GU. (n=6)

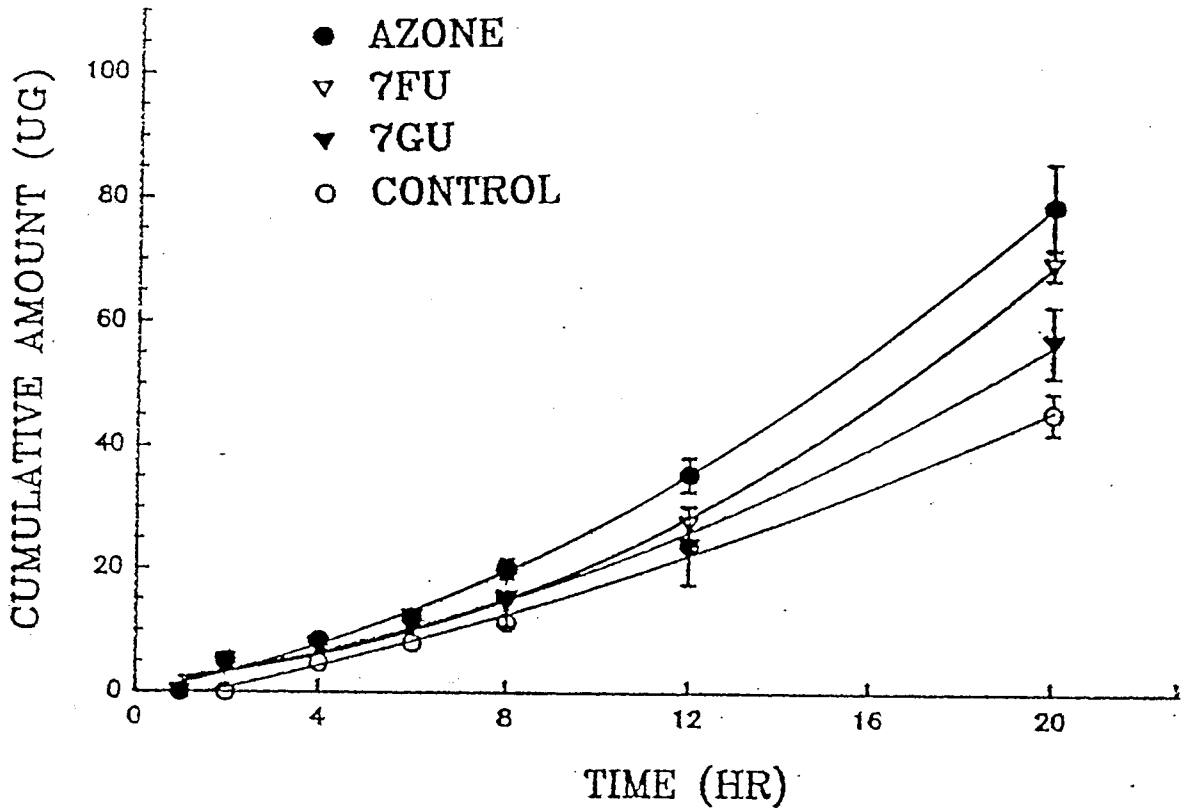


Figure I.10 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 9000 μg/ml ACV suspensions with vehicle Isoproyl Myristate in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; filled triangle, with 7GU. (n=6)

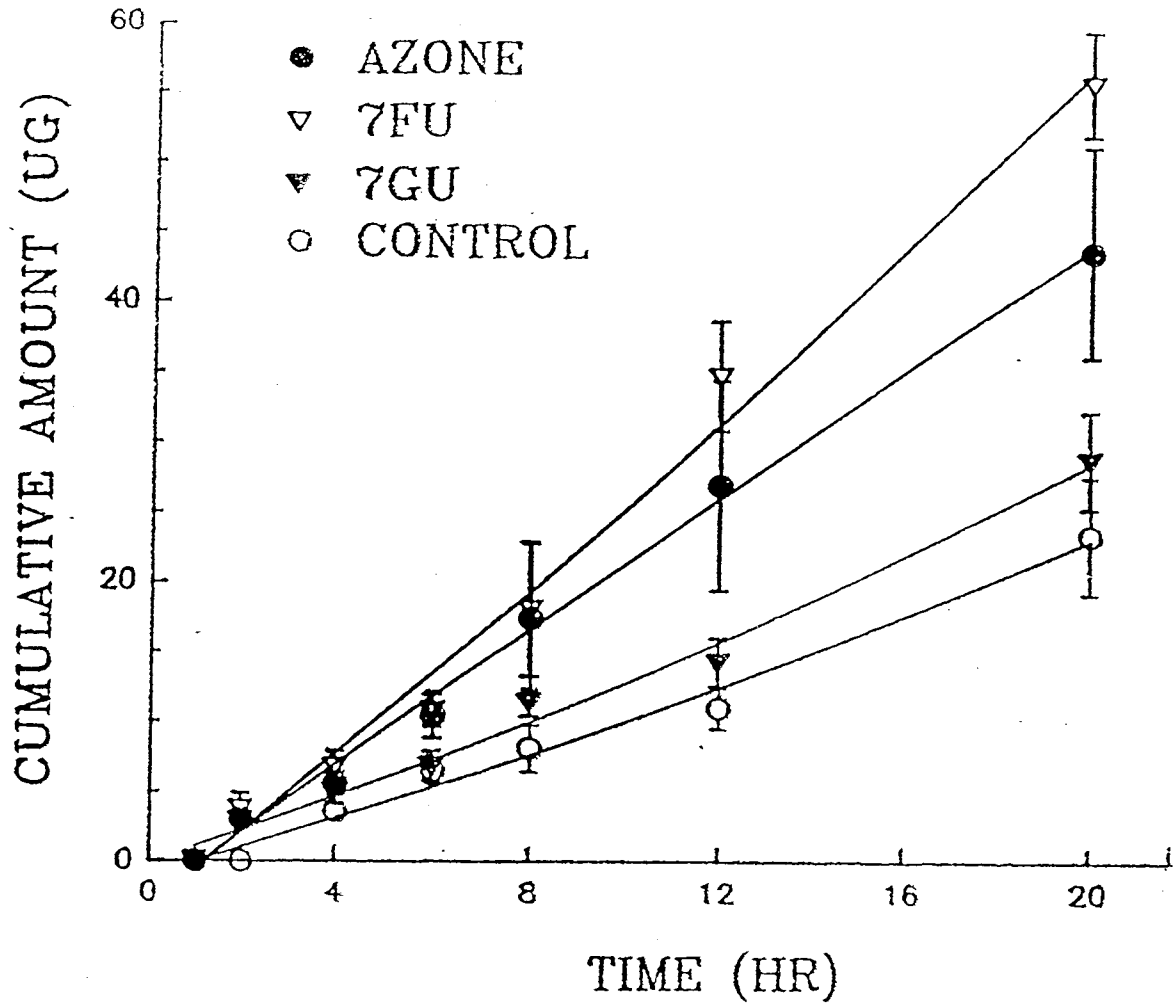


Figure I.11 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 5% ACV o/w creams with a 1% enhancer in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; filled triangle, with 7GU. (n=6)

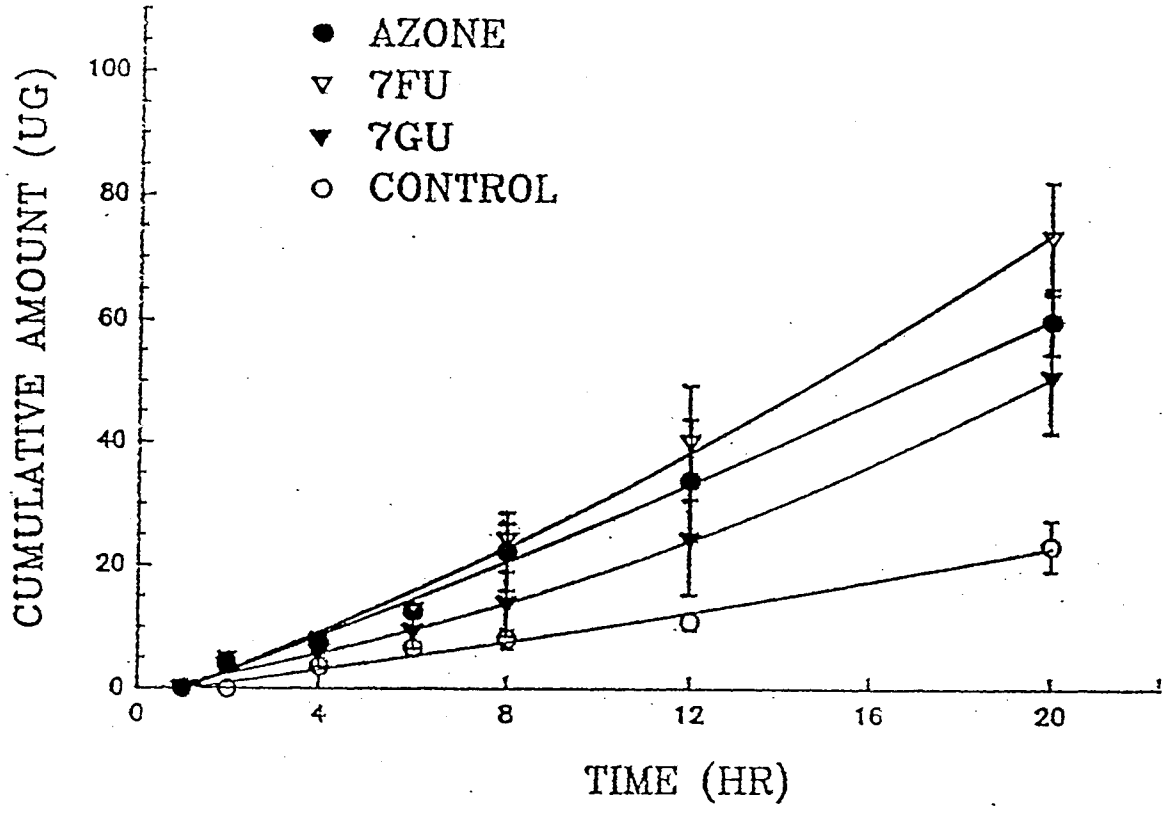


Figure I.12 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised chicken skin from 5% ACV o/w creams with a 2% enhancer in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; filled triangle, with 7GU. (n=6)

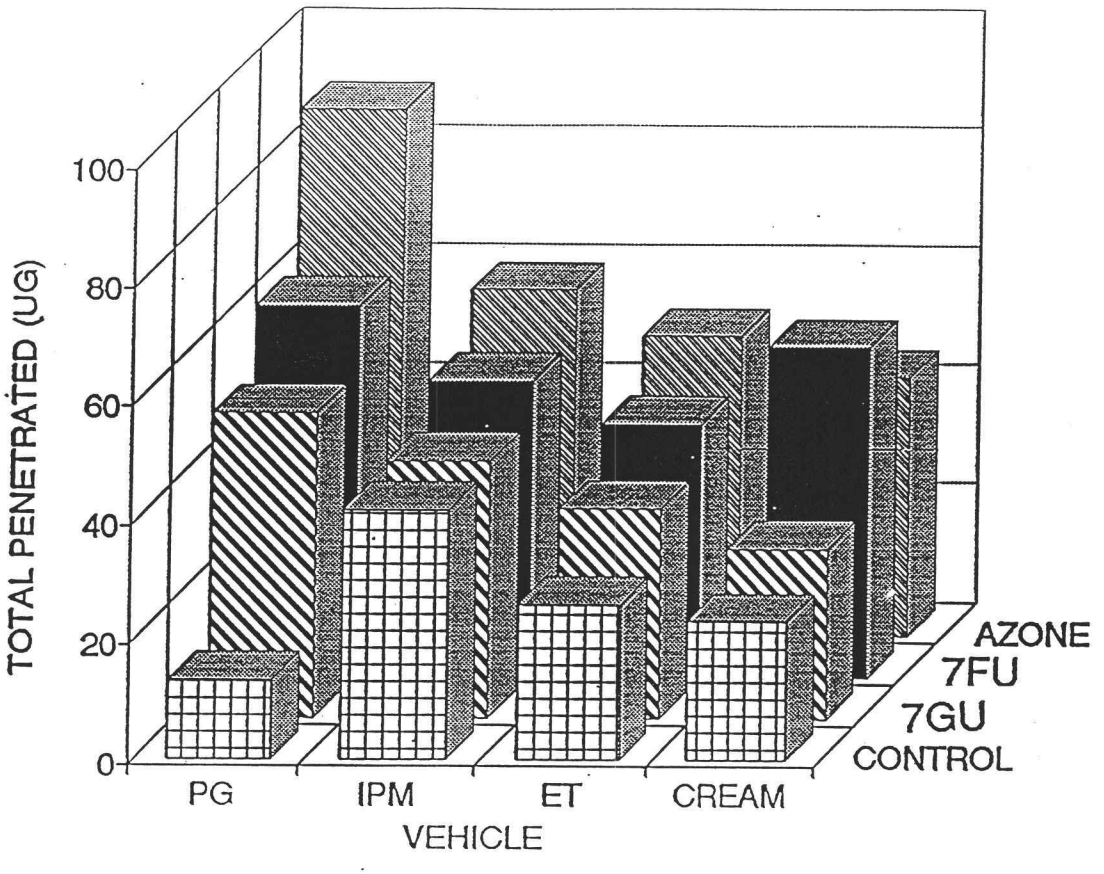


Figure I.13 Effect of vehicle and penetration enhancer on Acyclovir penetration: The total penetration of acyclovir through chicken skin from suspensions (ACV = 4500 μg/ml) and o/w creams (5% ACV) with or without a penetration enhancer (Enhancer 0.2M in a susupension, 1.0% in an o/w cream).

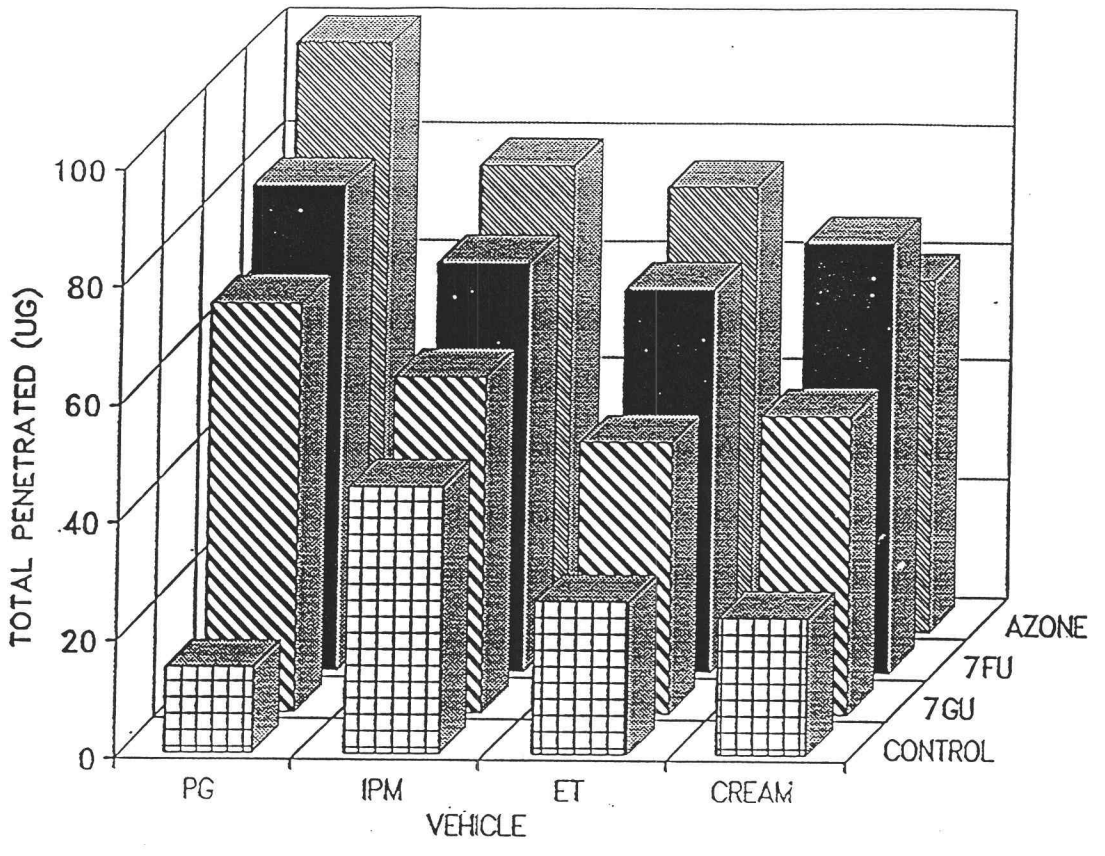


Figure I.14 Effect of vehicle and penetration enhancer on Acyclovir penetration: The total penetration of acyclovir through chicken skin from suspensions (ACV = 9000 μg/ml) and o/w creams (5% ACV) with or without a penetration enhancer (Enhancer 0.2M in a susupension, 2.0% in an o/w cream).

CONCLUSIONS

The percutaneous absorption of acyclovir from suspensions, ointments and o/w creams with or without the addition of penetration enhancer have been determined using a full-thickness lateral atperia fowl skin model. The effect of vehicles on ACV penetration without enhancer was: IPM > ET > PG in suspensions, and O/w cream >> Ointment. All selected penetration enhancers improved percutaneous absorption of ACV through avian skin from all applied topical ACV formulations. Azone provided the greatest enhancement from ACV suspensions, and maximum penetration was observed in solvent PG with an enhancer. The action of 7FU on ACV penetration from o/w cream ACV 5% was shown to be the most effective, especially when 2.0% 7FU was applied. Therefore, the best topical delivery medium for topical administration of acyclovir for treatment of herpes virus infections in chickens is a suspension of acyclovir in a vehicle of PG and an enhancer of Azone or a topical emulsion of a modified ACV o/w cream with 7FU.

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CHAPTER II

IN VITRO PENETRATION OF ACYCLOVIR THROUGH COCKATIEL SKIN MEMBRANES

INTRODUCTION

Pacheco's Parrot Disease (PPD) is a highly contagious, acute, and often fatal herpesviral infection which occurs in psittacine birds such as quaker parakeets and cockatiels (1). The signs of PPD usually follow a definite pattern. Yellowish, watery diarrhea occurs 1-2 days and depression only hours before death. When death occurs, it happens amazingly fast. Frequently there is no diarrhea, with death occurring acutely 2-4 hours after the warning sign of depression appears. Acute death is the hallmark of Pacheco's disease (2). Mortality rates can be as high as 100 percent in some species of psittacine birds (3). The herpesvirus of Pacheco's disease is shed in the feces and transmitted most often orally, through contaminated food, in contaminated water, or by preening contaminated plumage (2). There are two basic sources of Pacheco's virus. One is a shedding, sick bird before it dies. The other source is asymptomatic carriers which are totally resistant to the disease but harbor the virus. The incubation period for PPD is variable. The large psittacines can show sighs within 4½ days after exposure. On the other extreme, the incubation period may extend into years for other psittacines, with signs appearing when severe stress occurs (2).

Acyclovir (ACV) is an antiherpesviral compound which has low toxicity in uninfected host cells (3). The antiviral

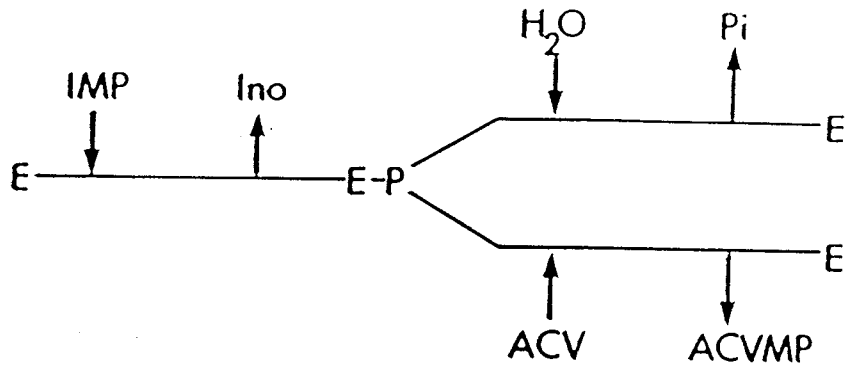

Figure II.1. Acyclovir phosphorylation by the cytoplasmic 5′ nucleotidase.

Mechanism: ACV interferes with DNA synthesis and inhibits viral replication.

effect of acyclovir on herpes simplex viruses and varicella zoster virus has been found to result from its interference with DNA synthesis and a selective inhibition of herpesvirus replication (3,4). Acyclovir has in vitro activity against some avian herpes viruses (5, 6, 7). IV or IM administration of acyclovir was applied in treatment of PPD in Quaker parakeets (8). It has also been reported to reduce the incidence of experimentally induced herpes virus infections (Marek's disease) in turkeys (9), but the topical ACV application in the treatment of herpesviral disease in avian species has not been reported yet. The poor In vitro and in vivo penetration of ACV from a topical 5% ACV ointment (commercially available) was reported using both human and guinea pig skin models (3,4), and clinical failure was observed in adequate ACV doses for therapy of herpesvirus disease after topical administration (4). To increase the penetration of topical ACV, various vehicles such as fatty acids (10), organic solvents {N-decylmethyl sulfoxide (11), Dimethyl sulfoxide (12), Ethanol, Isopropanol, Propylene glycol (13)}, a modified cream base (3,4), a transdermal delivery patch (16) and penetration enhancers {Azone (13), choline esters (15)} were used to improve percutaneous absorption of topical ACV administration (14).

From the results of the pilot (chicken) study, the greatest ACV penetration through fowl skin resulted from both PG/enhancer suspensions and modified (with enhancers) o/w

cream formulations. Moreover, the combination vehicle PG/Azone was shown as the best medium for an ACV suspension, and a cream/7FU base was the optimum for an ACV emulsion. In this chapter, the research objective was to study penetration of acyclovir from formulations which gave the greatest penetration in the pilot study through excised chicken skin membranes. The effect of vehicles and enhancers on percutaneous absorption of acyclovir from those topical formulations were re-evaluated in vitro using cockatiel skin so as to provide and explore the feasibility to treat herpes virus infection in psittacine species.

MATERIALS AND METHODS

Materials and Chemicals

Acyclovir was donated generously by Burroughs Wellcome Co., Research Triangle Park, NC; Azone (a gift) was obtained from Whitby Res. Inc., Richmond, VA; 7FU (1-farnesyl-ℰ-caprolactam) as a gift was provided from Kuraray Co., LID. Japan; Saline was obtained from Abbott Lab., North Chicago, IL; Propylene glycol and the o/w cream base components such as Sesame oil, Glycerol monostearate, Tween 80, Span 80, Trithanomine, Carbopol 940 were obtained from Merck & Co., Inc., Rahway, NJ; Hypoxanthine was provided from Sigma chemical Co., St. Louis, MO; Cockatiels were obtained from Department of Veterinary Medicine, Oregon State University, Corvallis, OR.

Preparation of Skin Samples

Ten cockatiels weighing 70-96 grams were sacrificed by cervical dislocation. Twenty pieces of the full-thickness lateral apteria skin samples were obtained after plucking feathers and removing adipose tissue, fascia and skeletal muscle from the undersurface. The excised skin was maintained at 25°C and moistened with saline solution continuously until used in the study. Vernier calliper (General Inc., USA) was

used to measure the thickness of cockatiel skin samples.

Experimental Design

The experiment was performed as a random design with three replications except for a PG/ACV suspension and 5% o/w emulsion with enhancer where two replications were used. All treatments were performed once due to limited skin samples. In this study, an organic solvent PG and an o/w cream base were selected as vehicles, and Azone and 7FU were added as penetration enhancers. ACV dose was 4500 μg/ml or 9000 μg/ml in a suspension, and 5% (w/w) in o/w creams. Details are listed in Table II1.

In vitro Diffusive Penetration Study

Percutaneous penetration of ACV was determined by an in vitro diffusion study. Samples were mounted on a diffusion cell with a surface area of 3.14 cm^2. 14 ml of saline solution was added into each receptor cell and stirred with a magnetic stirrer. Applied test formulations were (I) 20mM (4500 μg) or 40mM (9000 μg) ACV suspensions with solvent PG and 0.2M Azone or 7FU; (II) ACV o/w cream 5% with 2.0% Azone or 7FU. The 2.0 ml suspension or 1.0 gram of o/w cream was applied to the donor cell and the top sealed with parafilm. The diffusion cell was kept at 37°C (±0.5°C) by a circulating

water bath. Sampling times were chosen at 1, 2, 4, 6, 8, 12, and 20 hours after application. 0.8 ml of the receptor medium was withdrawn and was replaced with the same volume of fresh saline solution.

Assay of Acyclovir Penetration

Acyclovir assays were carried out with a high performance liquid chromatograph (HPLC) (M-15 Waters Associate Inc., MA) which was equipped with a 5-micron C18 column (Phenomenex Inc., CA) and UV detector (m-440 Waters Associate Inc., Milford, MA) operating at 254nm. The mobile phase was a mixture of methanol and distilled water (10:90), at a flow rate of 0.9 ml/min. Before analysis, samples obtained from the receptor cell were filtered through a 5-μm filter needle and centrifuged 8 min at 9000 rpm (or 760 g force) (Centrifuge 5415 C, Brinkmann Instruments Inc., Germany). Quantitation was performed using peak area measurements for acyclovir in the diffusate. Hypoxanthine 5.0 μg/ml was employed as the internal standard.

Table II.1 Experimental design for ACV penetration study using cockatiel skin model

Formula	Vehicle	ACV Dose (μg/ml, %)	Enhancer (0.2M)	Treatments (n)
1	PG	4500	-	2
2	PG	4500	Azone	3
3	PG	4500	7FU	3
4	PG	9000	Azone	3
5	PG	9000	7FU	3
6	Cream Base	5%(w/w)	Azone	2
7	Cream Base	5%(w/w)	7FU	2

RESULTS AND DISCUSSION

(I) Suspension Percutaneous absorption of acyclovir through cockatiel skin from PG solvent with or without the penetration enhancer is shown in Table II.2, and the penetration of ACV vs time profiles are illustrated in Figure II.2 and II.3. A significant difference in penetration of ACV was observed between ACV suspensions with a solvent PG and PG with penetration enhancer ($P<0.05$). Cumulative penetration of ACV from a 20mM ACV/PG suspension was increased 26 fold by the presence of penetration enhancer Azone, and 13 fold by 7FU over ACV in PG solvent alone (Figure II.3). ACV penetration from suspension with enhancer Azone was enhanced by 51.40% (20mM) and 42.62% (40mM) above the enhancement achieved by 7FU. Doubling the amount of ACV in the PG/enhancer suspension from 20mM to 40mM caused the total ACV penetration to increase 43.3% to 51.98% for Azone and 7FU, respectively (Figure II.2, II.3). The enhancement may mainly be attributed to the binary vehicle with an organic solvent and a penetration enhancer by their improving the physicochemical properties of the skin to penetration of acyclovir or altering the solubility of ACV, or both (13, 14). Compared to effects of penetration enhancers, Azone provided the greater improvement than 7FU from PG/enhancer suspensions, therefore, the option of Azone as a ACV promoter was preferable. ACV penetration using cockatiel skin model was observed to be much greater in extent compared

to penetration of chicken skin membrane, but the tendency of penetration was similar. Diffusion equation (17) indicates that the rate of penetration is inversely proportional to thickness of the skin. The greater ACV penetrating through cockatiel skin may partly be due to the difference in thickness of cockatiel skin (h=0.04cm) compared to the thickness of chicken skin membrane (h=0.12cm). Studies have demonstrated that percutaneous absorption appears to be greater when the drug is applied to skin with a thin horny layer than with one that is thick due to the properties of the stratum corneum difference in number of cell layers, stacking of cells size, and amount of surface lipid (14). Therefore, ACV penetration variation between chicken and cockatiel skin maybe mainly attributed to different thicknesses of skin in the two species.

(II) O/w cream A comparative experiment between the effect of enhancer (2.0%) Azone and 7FU was performed by in vitro diffusion using cockatiel skin membranes. The analytical results are reported in Table II.3. ACV penetration from a o/w cream with 7FU was 57.76% higher than that with Azone (Figure II4). The ability of 7FU to increase ACV penetration from o/w cream was 3.87 times greater over the extent of improvement in ACV penetration by Azone (Figure II4).

Reviewing results of vehicle effects observed in Chapter I, acyclovir penetration through chicken skin from suspension

with IPM (δ=8.54) was the greatest, however, the highest improvement occurred in presence of PG solvent (δ=14.8) with enhancer Azone (δ=9.07). Azone effect in ACV suspensions was shown stronger with a hydrophilic vehicle than with a lipophilic medium, while, the action of 7FU on o/w cream was the most important. The o/w cream base was formulated by thirteen kinds of compounds (Table II.4) in an oil-in-water combination type, ACV may dissolve in both oil and water phases in a different extent, thus, the action of enhancers on this combination vehicle maybe different from that in an unique phase in a single vehicle like solvent PG. 7FU was shown as the best in o/w cream formulations, probably because it had greater effect than Azone on both parts (oil and water phase) of the combination vehicle.

The major reason for the alteration in ACV penetration across species was due to variation of the thickness of skins between chickens and cockatiels (14).

Table II.2 Cumulative penetration of acyclovir (μg) through cockatiel skin from ACV/PG suspensions with or without 0.2M enhancers

TIME		DOSE 4500μg/ml	
	CONTROl (n=2)	+AZONE (n=3)	+7FU (n=3)
1	0.000	14.84±3.661	6.020±1.372
2	2.800±1.980	32.85±10.85	12.26±0.277
4	5.320±0.792	81.48±20.69	20.44±0.792
6	7.210±0.693	129.6±11.34	46.55±17.72
8	8.740±0.434	192.7±41.29	71.84±20.58
12	9.870±1.484	229.5±33.28	119.8±12.67
20	15.12±0.594	390.9±23.37	190.0±32.07
		DOSE 9000μg/ml	
		+AZONE (n=3)	+7FU (n=3)
1		73.64±9.240	31.36±15.42
2		155.4±29.13	47.04±17.53
4		218.0±12.45	77.90±25.28
6		286.3±10.65	112.7±25.06
8		387.7±11.17	196.2±36.44
12		522.6±31.46	242.2±39.20
20		689.6±25.34	395.7±17.90

Table II.3 Cumulative penetration of acyclovir (μg) through cockatiel skin from o/w cream 5% with 0.2M enhancers

TIME (HR)	+AZONE (n=2)	+7FU (n=2)
1	9.030±2.079	30.94±1.284
2	11.06±3.168	45.78±1.582
4	21.98±4.357	63.21±9.212
6	32.62±6.038	95.41±20.09
8	45.78±7.326	127.9±10.79
12	64.96±3.959	194.0±7.920
20	120.1±8.414	282.2±18.41

Table II.4 Emulsion bases of topical ACV formulations

	Materials	Percentage
1.	O/W Cream base:	
A.	Sesame oil	6.0
	Glycerol monostearate	2.0
	Tween 80	1.5
	Span 80	0.5
	Isopropyl myristate	2.0
	Triethanolamine	0.5
B.	Distilled Water	75.5
	Propylene glycol	6.0
	Carbopol 940	0.75
	Ethanol	5.0
	Preservative	0.3
2.	Ointment base:	
	Polyethylene glycol	100

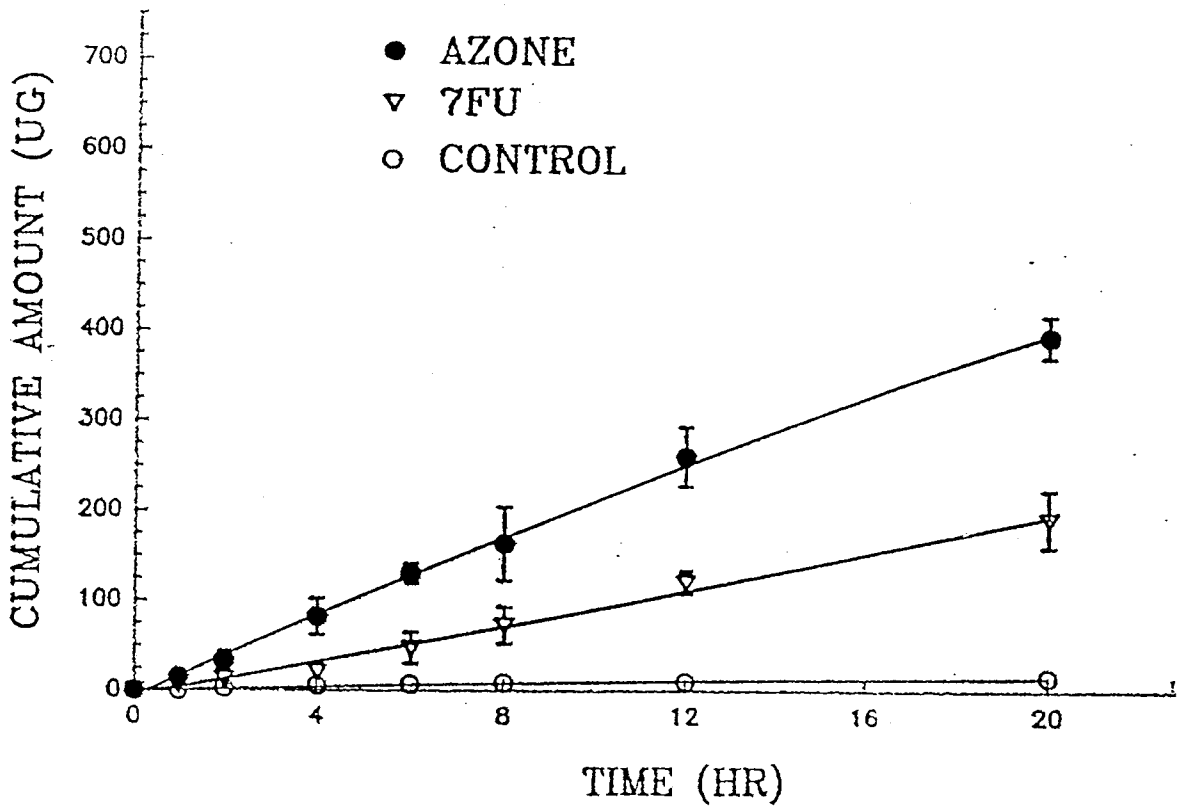

Figure II.2 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised cockatiel skin from 4500 μg/ml ACV suspensions with vehicle Propylene Glycol in 20-hour diffusion study in vitro. Open circle, with no enhancer; filled circle, with Azone; open triangle, with 7FU; (n=3)

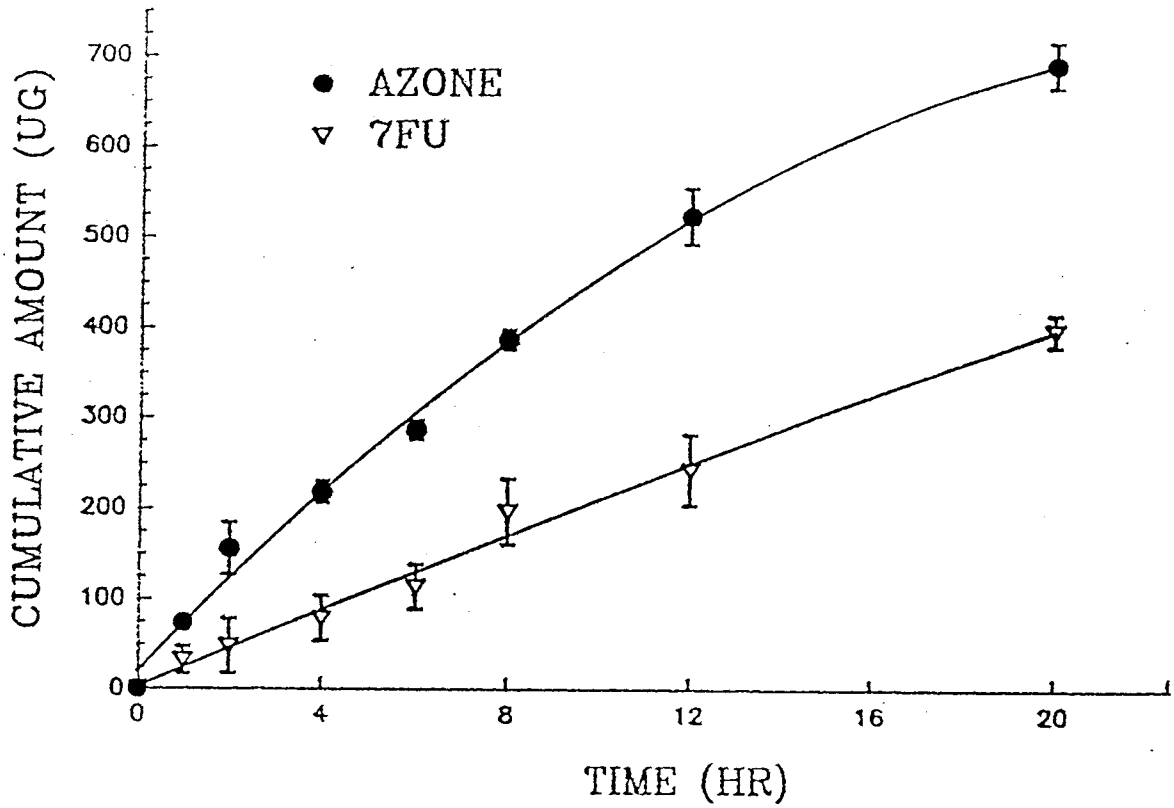

Figure II.3 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised cockatiel skin from 9000 μg/ml ACV suspensions with vehicle Propylene Glycol in 20-hour diffusion study in vitro. Filled circle, with Azone; open triangle, with 7FU; (n=3)

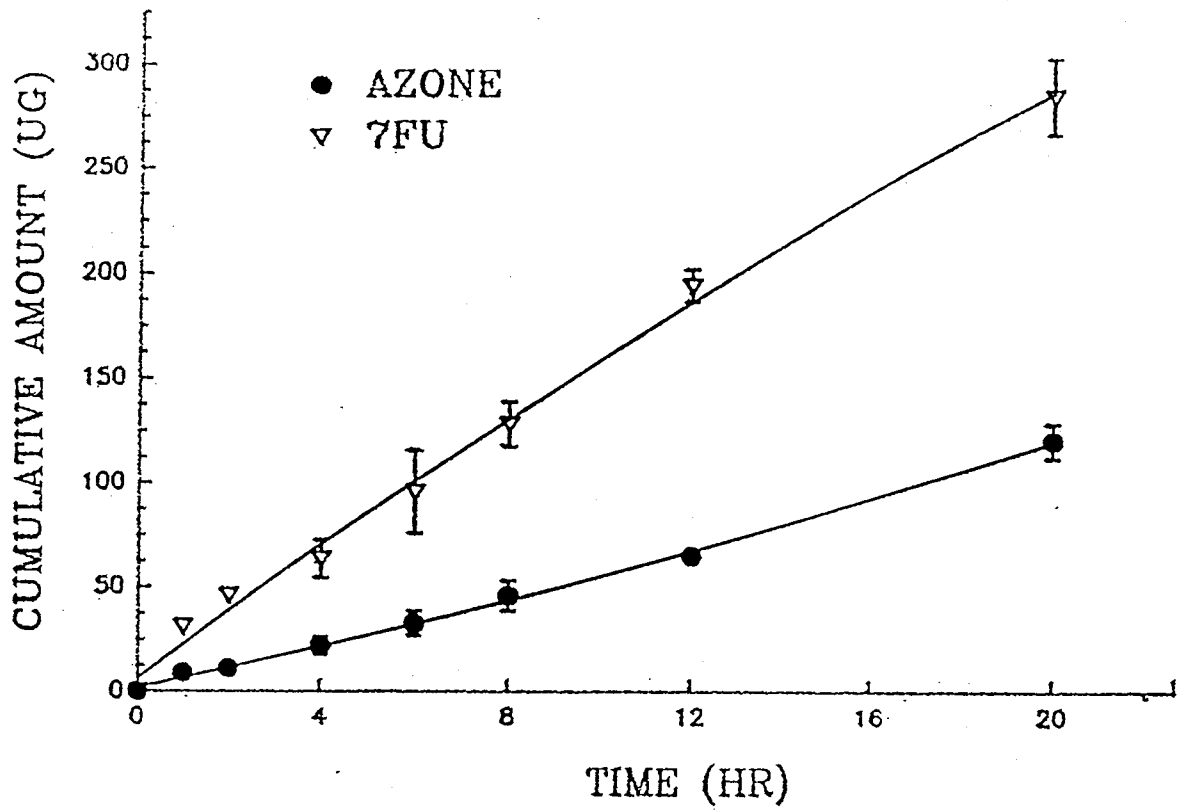

Figure II.4 Effect of penetration enhancer on Acyclovir penetration: Cumulative penetration profile of acyclovir through excised cockatiel skin from 5% ACV o/w creams with a 2% enhancer in 20-hour diffusion study in vitro. Filled circle, with Azone; open triangle, with 7FU; (n=2)

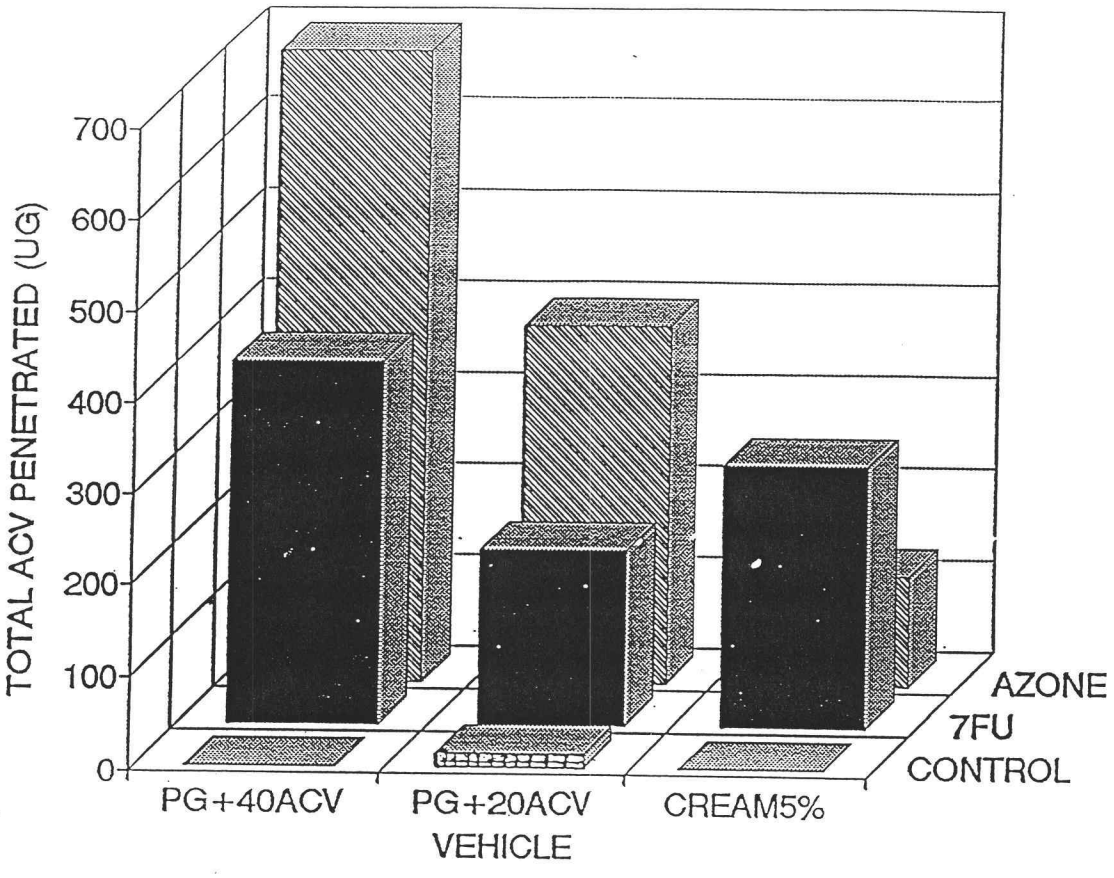

Figure II.5 Effect of vehicle and penetration enhancer on Acyclovir penetration: The total penetration of acyclovir through cockatiel skin from PG suspensions (ACV dose: 40mM [9000 μg/ml] or 20 mM [4500 μg/ml]) and 5% o/w creams with or without a penetration enhancer (Enhancer 0.2M in a susupension, 2.0% in an o/w cream).

CONCLUSIONS

Percutaneous absorption of acyclovir by *in vitro* diffusion through cockatiel skin membranes was determined. ACV penetration from a suspension containing the solvent PG was dramatically improved by addition of a penetration enhancer, either Azone or 7FU. Azone was shown more effective than 7FU in increasing passage of ACV through cockatiel skin membrane from its PG suspension. Also, in the PG solvent with an enhancer, ACV penetration increased with increasing ACV present in the suspension. For ACV o/w cream formulations, the effect of 7FU was greater than Azone in increasing penetration of ACV across cockatiel skin membrane.

A 40mM ACV suspension in PG and 0.2M Azone, or a 5% ACV in an o/w emulsion cream base with 2.0% 7FU may provide sufficient release and penetration of ACV for treatment of herpes virus infection in cockatiels. Such a study should be carried out to verify these conclusions.

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