1994

SYSTEMATIC STUDIES TO EVALUATE THE EFFECTS OF VEHICLES ON THE *IN VIVO* DRUG DELIVERY TO THE SKIN

Sudhanva R. Marathe
University of Rhode Island

SYSTEMATIC STUDIES TO EVALUATE THE EFFECTS OF VEHICLES ON THE *IN VIVO* DRUG DELIVERY TO THE SKIN

BY

SUDHANVA R. MARATHE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

PHARMACEUTICAL SCIENCES

UNIVERSITY OF RHODE ISLAND

1994

MASTER OF SCIENCE THESIS

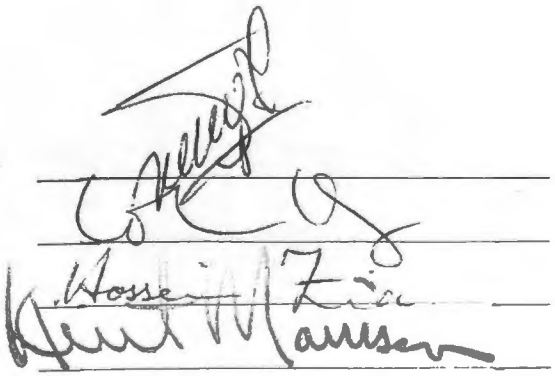
OF

SUDHANVA R. MARATHE

APPROVED:

Thesis Committee

Major Professor



DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

1994

ABSTRACT

Current trend in evaluating dermatological formulations is shifting towards measuring the **retention** of drugs in the skin rather than **flux** through the skin. This study was undertaken to emphasize the importance of drug retention and to investigate possible factors which affect it.

An *in vivo* hairless rat model was used in this study to evaluate the effect of **26** vehicles on the local drug delivery to the skin layers. For this purpose, two model drugs, ^{3}H-Hydrocortisone (HC) and ^{3}H-triamcinolone acetonide (TAC), were used. Vehicles selected belonged to seven different classes including glycols, polyglycols, alcohols, esters, glycerides, mixed commercial vehicles and enhancers.

Solubilities of HC and TAC were determined in these vehicles using an HPLC method. The solubility data showed large ranges, 1912 fold for HC and 2297 for TAC, indicating a need to evaluate the influence of solubility on the skin uptake of these drugs.

In vivo retention studies were carried out in the hairless rat model using the "finite dose" approach. The drug uptake in the tissue was expressed in terms of μg of drug per gm of tissue and percent of applied dose retained in the skin layer. These data were then categorized into various classes of the

vehicles used and the following findings were observed:

- Retention of HC and TAC in the epidermis and dermis was affected dramatically by the choice of vehicles.

- The overall rank orders and ranges were different for epidermal and dermal retention for both drugs indicating that the vehicle effects are different for the epidermis and dermis.

- In most cases the rank orders and ranges within different classes of vehicles was different for both drugs.

- Ratios of epidermal to dermal retention ranked differently for HC and TAC.

The foregoing observations e.g. differing rank orders and ranges, lead us to recommend the use of **retention** data instead of **flux** data in screening vehicles for optimizing dermatological formulations for these two drugs.

ACKNOWLEDGEMENTS

A number of people have been instrumental in making this research study possible and a success.

First of all, I would like to thank my advisor Prof. M. S. Kislalioglu for her faith in me for doing this research. She has steered me through times when I needed her professional guidance and mentorship. She was always supportive and enthusiastic about trying out innovative ideas for the research study. I would like to thank her for her patience and assistance during the entire span of the research. I am also thankful to the Society of Cosmetic Chemists for providing me with a grant in the first year of the program.

I would like to thank Dr. Needham and Dean Luzzi for allowing me to stay off campus for continuing the research. Their guidance, support and suggestions are appreciated.

I would like to thank Dr. Kelly for providing me invaluable advice during certain phases of the research. Also I am very thankful to Dr. H. Zia for giving me valuable suggestions and assistance during the period of research. Thanks are also due to Dr. C. Lee for his valuable suggestions and constructive criticisms.

I am grateful to Dr. A. W. Malick for providing me the opportunity to conduct the research at Roche Laboratories. His mentorship, guidance and suggestions have been important and useful. Major credit for selecting this timely research of practical applications goes to him. I will always remain indebted to him for the valuable research experience which I gained in an industrial environment.

I would also like to extend my gratitude to Dr. Charan Behl for providing me invaluable assistance and close cooperation during this research. He was understanding, patient and very helpful during all the phases of this research study. I have learned a lot from his operational techniques. He was innovative in implementing different ideas and theories during this study. He provided constant support and counselling whenever I encountered any problems during the whole study.

I wish to express my sincere thanks to Ms. A. J. Scheer, Mr. M. Yelvigi and Mr. C. Sunwoo for making it possible for me to start my career at Roche. Their valuable suggestions, encouragements and guidance are truly appreciated.

Thanks are also due to Dr. H. K. Sandhu, Dr. S. Gunturi and Dr. F. Qureshi for helping me out in some difficult moments in the preparation of this thesis. I would like to thank Dr. S. B. Patel and Mr. D. Piemontese in teaching me the analytical and animal handling techniques during the primary phases of the study. I would like to thank Dr. S. Kumar and Dr. H. Char in helping me to understand some software programs.

I would like to thank Anand, Sumeet, Raghu, Poly, Ketan, Rose, Jatin, Mark and Kathy, in helping me out during some difficult times. Without their help it would have been very difficult to achieve the completion of this thesis.

The financial support for this research was made possible from a research grant from Hoffmann-La Roche, Inc., Nutley, NJ.

PREFACE

This thesis has been written in a standard format. It consist of a systematic study to evaluate the effect of vehicles on the *in vivo* drug delivery to the skin layers. It has been subdivided into seven sections.

The first section introduces the subject of topical drug delivery and the effect of vehicles. This is followed by a section on objectives. The experimental techniques are discussed in the third section. The results obtained are discussed in the fourth section. The entire work is summarised in the fifth section. References are in the sixth section followed by appendices which consist of raw data tables. The last section consists of bibliography.

TABLE OF CONTENTS

		Page
1.0	**INTRODUCTION**	1
	1.1. Properties of Skin Relevant to Drug Delivery	3
	1.2. Definitions Pertaining to the Transport of Drug To and Through the Skin	11
	1.3. Assessment of Skin Permeation and Retention	13
	1.4. Factors Which Affect Drug Delivery Into and Through the Skin	25
2.0.	**OBJECTIVES**	47
3.0.	**EXPERIMENTAL**	48
	3.1. Materials	48
	3.2. Methods	50
	3.3. Data Analysis	58
4.0.	**RESULTS AND DISCUSSION**	59
	4.1. High Performance Liquid Chromatography Method	59
	4.2. Solubility of Hydrocortisone and Triamcinolone Acetonide in Vehicles Studied	59
	4.3. *In Vivo* Retention Procedures	61
5.0.	**SUMMARY AND CONCLUSIONS**	89
6.0.	**REFERENCES**	93

TABLE OF CONTENTS (Cont'd)

		Page
APPENDIX A:	**A.1. - A.26.** Tables of Epidermal and Dermal Retention Data For Hydrocortisone From Different Vehicles Studied	112
APPENDIX B:	**B.1. - B.26.** Tables of Epidermal and Dermal Retention Data For Triamcinolone Acetonide From Different Vehicles Studied	139
7.0	**BIBLIOGRAPHY**	166

LIST OF TABLES

		Page
1.	List of Animal Models Used in Transdermal and Dermatological Drug Delivery	17
2.	Ranking of different Vehicles According to the Flux of Estradiol	32
3.	List of Some of the Most Commonly Used Marketed Dermatological Products	34
4.	Drugs That Exhibit Azone-Induced Permeation Enhancement	43
5.	Instrumentation and Experimental Conditions for HPLC Analysis of Hydrocortisone and Triamcinolone Acetonide	52
6.	Solubility of Hydrocortisone and Triamcinolone Acetonide in the Vehicles Tested	60
7.	Ranking of Vehicles, Classwise, According to the Percent Dose of Hydrocortisone Retained in the Hairless Rat Skin Layers	62
8.	Ranking of Vehicles, Classwise, According to the Percent Dose of Triamcinolone Acetonide Retained in the Hairless Rat Skin Layers	63
9.	Ranking of All Vehicles Tested According to the Percent of Dose of Hydrocortisone Retained in the Hairless Rat Skin Layers	70
10.	Ranking of All Vehicles Tested According to the Percent Dose of Triamcinolone Acetonide Retained in the Hairless Rat Skin Layers	71
11.	Ratios of Epidermal to Dermal Retention of Hydrocortisone from Different Vehicles Tested	86
12.	Ratios of Epidermal to Dermal Retention of Triamcinolone Acetonide from Different Vehicles Tested	87
A.1. to A.26	Tables of Epidermal and Dermal Retention Data For Hydrocortisone From Different Vehicles Studied	113
B.1. to B.26	Tables of Epidermal and Dermal Retention Data For Triamcinolone Acetonide From Different Vehicles Studied	140

LIST OF FIGURES

		Page
1.	Schematic Representation of the Structure of Epidermis	5
2.	Detailed Representation of the Microscopic Structure of the Skin Layers	10
3.	Schematic Representation of Local Events After Application of a Transdermal or Dermatological Formulation	15
4.	Linearity Plot of Peak Area vs Concentration for Hydrocortisone and Triamcinolone Acetonide ranging from 1 μg/mL to 10 μg/mL	55
5.	Linearity Plot of Peak Area vs Concentration for Hydrocortisone and Triamcinolone Acetonide ranging from 0.015 mg/mL to 1 mg/mL	56
6.	Plots of Retention vs Solubility for the Class of "Glycol" Vehicles Studied	74
7.	Plots of Retention vs Solubility for the Class of "Polyglycol" Vehicles Studied	75
8.	Plots of Retention vs Solubility for the Class of "Alcohol" Vehicles Studied	76
9.	Plots of Retention vs Solubility for the Class of "Ester" Vehicles Studied	77
10.	Plots of Retention vs Solubility for the Class of "Glyceride" Vehicles Studied	78
11.	Plots of Retention vs Solubility for the Class of "Commercial" Vehicles Studied	79
12.	Plots of Retention vs Solubility for the Class of "Enhancer" Vehicles Studied	80

1. INTRODUCTION

Drug substances are administered through various routes to obtain either systemic or local therapeutic effects. For systemic effects, drugs are delivered into the blood stream by administering them via the parenteral, oral, nasal, ocular, vaginal, and rectal routes. Recently, the skin has been used for the systemic drug delivery by using transdermal products. For local effects, drugs are applied on the surface of the affected tissue. Topical products include ophthalmic, nasal, auricular, buccal, vaginal, dermatological, and rectal products. These products can be apparently termed as targeted drug delivery systems because they provide drugs at the site of action. All products designed to provide local therapeutic effects can be termed as topical products.

The principles involved in transdermal and dermatological products have often been mistakenly used interchangeably. Transdermal products are designed to provide maximum transport of drugs across the skin into the blood stream with no or minimal drug retention in the skin layers. Dermatological products on the other hand are designed with an opposite purpose, i.e. to obtain a maximal drug retention in the skin layers with no or minimal drug transport across the skin in the blood stream. Thus, different pharmacokinetic principles are involved in these two types of drug products. Besides

pharmacokinetic principles, the formulation principles involved are also quite different.

Although dermatological products have been in existence for tens of decades, the mechanistic aspects of drug delivery to the skin layers and scientific principles to optimize it, and predictive selection of optimal vehicles/excipients have not been well studied and understood. This is true mainly because the emphasis has long been placed on the optimization of cosmetic/aesthetic and toxicological properties of dermatological products. Selection of vehicles/excipients was based primarily on these two parameters. Until quite recently, the critical importance of optimal drug delivery to the skin and its relation to therapeutic effect was all but ignored.

Principles involved in the design of transdermal products have been studied for over two decades and are well understood. As the momentum in the development of transdermal products increased, researchers began to realize the importance of local drug delivery for dermatological products. But, unfortunately, this was done by using scientific principles applicable to transdermal products. Awareness of this issue became clear at two recent international workshops [1,2] on the optimal development of dermatological drug products.

Optimal local drug delivery from dermatological products is

vital to obtain desired therapeutic effects. To accomplish this, selection of formulation vehicles/excipients is critically important. It is necessary to optimize local drug delivery prior to testing formulations in the clinic. If desired efficacy is not obtained, further efforts should be made to improve drug delivery. The improved formulation (s) should be tested again in the clinic. This feed-back cycle would allow a proper interpretation of clinical data and development of an optimal product.

1.1. Properties of Skin Relevant to Drug Delivery:

Skin is the most exposed and the largest organ in the human body. It is also a complex and sophisticated organ we do not know much about. The origin and the causes of many dermatological diseases are not understood. The precise targets in the skin layers responsible for ailments and site of action of drugs are usually not known. It is quite a challenge for a pharmaceutical scientist who develops formulations and the dermatologist who uses it on patients.

The major function of the skin is that of a protective organ. It is supposed to, among other functions, protect the body against environmental hazards by keeping harmful chemicals from absorbing through the skin into the systemic circulation. It also prevents water loss from the body, a function which is

vital to the human survival[3]. The skin is not meant for the intake of substances. The objectives involved in developing either transdermal or dermatological products are against this property of the skin, i.e. to overcome the natural barrier properties of the skin.

The skin is a multilayered organ[3]. The outermost layer, stratum corneum, together with the underlying viable epidermis, functions as a protective layer preventing water loss as well as the absorption of foreign matter from the environment. The stratum corneum plus the viable epidermis can also be called "epidermis". The dermal components also provide elasticity and flexibility to the skin. Together, the stratum corneum, viable epidermis and particularly the dermis provide a firm and durable envelop which coupled with the underlying fatty tissues give form and necessary structure and flexibility to the skin.

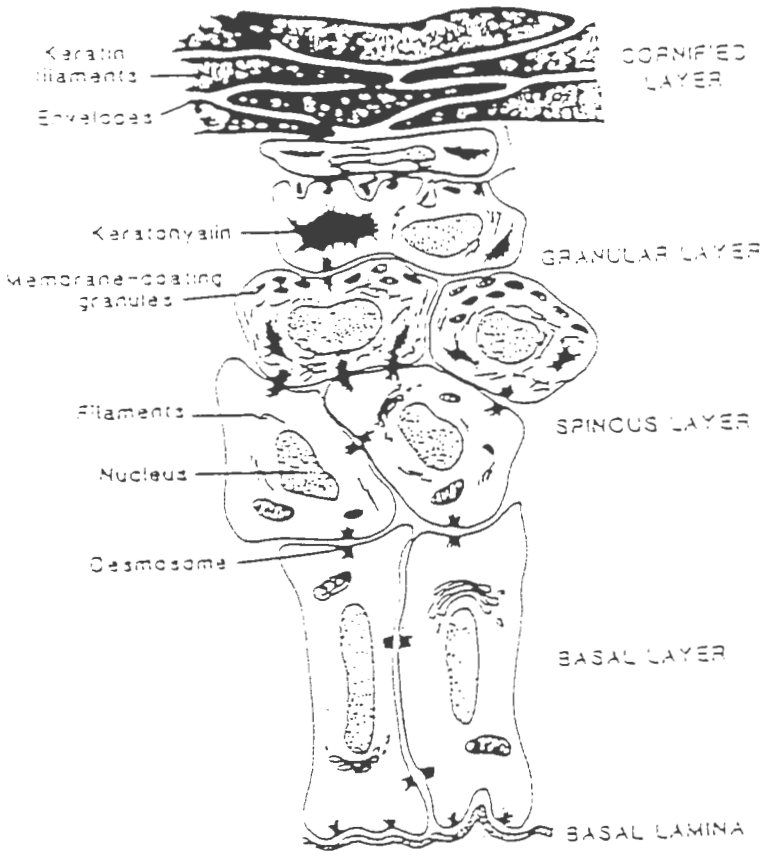


Figure 1 Schematic Representation of the Structure of the Epidermis (**Adapted from R. L. Eckert, 1992**)[4]

1.1.1. Epidermis: The upper layer of the skin is called as the epidermis[5]. The basal cell layer (Stratum Germinativum) comprises 90 % of the epidermal cells. This layer is responsible for the formation of the protective layer (Stratum Corneum) which provides a barrier to the uptake of pathogens, prevents water loss and is abrasion resistant. The basal cells undergo a programmed process of cell division in which the undifferentiated cells are converted to highly differentiated, non-living cells. Basal cell proliferation is regulated by a variety of intrinsic and extrinsic factors which are not fully understood[4]. The spinous layer (Stratum Spinosum) is situated directly above the basal cells. These cells are biochemically distinct in the sense that they synthesize a different set of macromolecules[6]. This layer is called as the spinous layer because of its spiny like appearance. The granular layer (Stratum Granulosum) consists of living cells. These cells contain electron-dense keratohyalin granules that contain profilagrin. The transition zone (Stratum Lucidum) is situated between the granular and cornified layers. It is a transition zone between living and dead epidermis. This is a zone of excessive cellular remodeling. Most of the existing cellular organelles, DNA and RNA, are destroyed by the activity of proteases and nucleases. The lipid contents of the membranes coating granules are released into the extracellular matrix, the keratin filaments are restructured to a more stable form and the cornified envelop is formed. The cornified layer

(Stratum Corneum) is the last stage of keratinocyte differentiation and is made up of corneocytes. The corneocytes are shaped as flattened polyhedrons and are held together by modified desmosomes and an interlocking system of ridges and grooves. The major protein component of the corneocyte is keratin structured into macrofibrillar bundles. Another major component is the cornified envelop which consists of a covalently crosslinked sheet of protein. This layer also contains various lipids on the surface formed by the excretion of sebum emanating from the sebaceous glands which help in protecting the body against environmental chemicals/substances. The stratum corneum of various species including murine, porcine, rodent and humans was studied and it was concluded that the majority of lipids consisted of sphingolipids. Other lipid components of relatively lesser importance include cholesterol, triglycerides and fatty acids[7,8,9].

Two other cell types which populate the epidermis are the Merkel cells and the Melanocytes. The function of Merkel cells is not well understood, although it is believed that these cells contain neurotransmitters and may function as a communication point between surrounding keratinocytes and neurons. Melanocytes are pigment producing cells which originate in the neural crest and are distributed among the basal keratinocytes. Melanin produced in melanosome and

featured in melanocytes provides the skin color[4].

1.1.2. Dermis: The dermis comprises the largest fraction of the skin and is responsible for providing its structural strength. It consists of elastin which serves as the connective tissue. It also provides an environment for nerve and vascular network and appendages needed to support the epidermis. The major dermal cell types are fibroblasts, macrophages, and mast cells, although other cell types, e.g. lymphocytes, plasma cells, etc. frequently populate the dermis in response to injury and other stimuli. The fibroblast is responsible for the synthesis and destruction of the connective tissue proteins. Fibroblasts secrete collagen and elastin which in turn secrete collegenase and gelatinase which remodel collagen fibrils[10]. Macrophages originate as precursor cells in bone marrow, mature into monocytes in the blood, and terminally differentiate to macrophage in the tissue. Their main function is to process and present antigens to immunocompetent lymphoid cells. They also synthesize and secrete interleukin 1 (IL-1), growth factors, prostaglandins, and interferon[6]. Mast cells are present in all layers of the dermis with focal concentration around blood vessels. They produce granules full of histamine which is vasoactive. Mast cells respond to light, cold, acute trauma, vibration, and pressure, as well as chemical and immunological stimuli. When

triggered by these stimuli, they release histamine from the granules initiating irritation and/or sensitization[11].

In Figure 2, a schematic and more complete structure of the skin is displayed[4]. In addition to the epidermis and dermis, this structure shows hair follicle, hair shaft, sebaceous gland, dermal and subcutaneous vasculature, and eccrine gland. Besides their pharmacological significance, these structures also play an important role in defining drug delivery pathways.

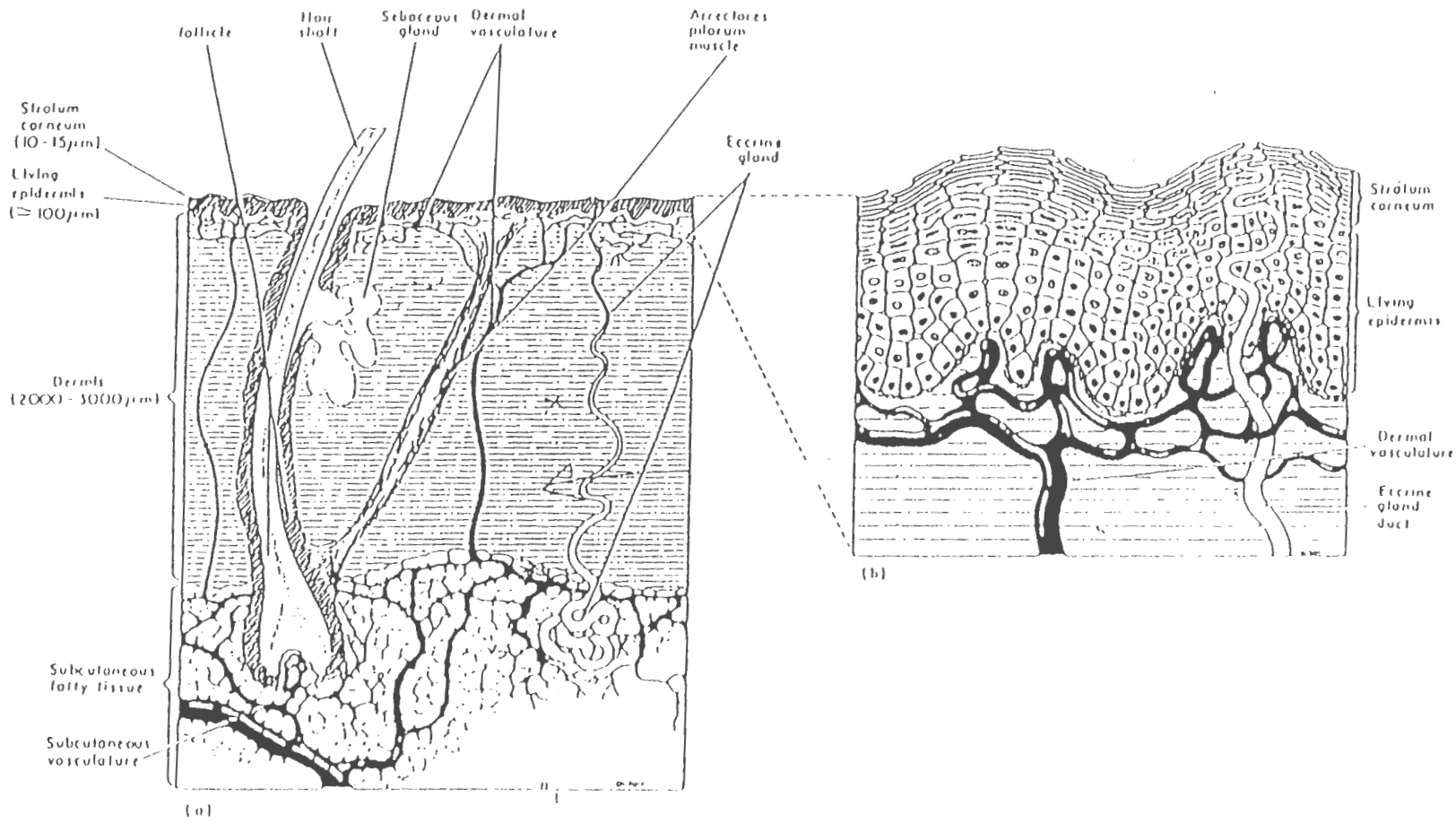


Figure 2. Detailed Representation of the Microscopic Structure of the Skin Layers (Adapted From Eckert, R. L., 1992)[4].

1.2. Definitions Pertaining to the Transport of Drug To and Through the Skin

Permeation is defined as net transport of drugs across the skin into the systemic circulation[12]. This term is relevant to transdermal products where formulation is applied in an infinite (reservoir) dose manner. Other comparable terms are **absorption** and **transport**. Permeation has been expressed quantitatively either as percent absorption, percent permeated, or permeability coefficient[13]. The last term is often used in describing transdermal drug delivery. Percent absorption is not a meaningful expression for transdermal products. One can carry out in vivo or in vitro transdermal experiments in order to optimize formulations. Fast permeation and clearance are desired features for an optimal product. In vivo animal studies are carried out by applying the formulation in a patch to the abdominal skin of the model animal used. The drug transport across the skin is calculated by measuring the blood levels for the drug. In vitro studies are carried out by placing a piece of the model skin between two halves of a diffusion cell. Various diffusion cells have been designed and modified to provide accurate determinations of penetrant flux[14]. The upper half of the diffusion cell is called as the "donor" compartment. The formulation to be tested is placed in this compartment. Since a reservoir of the formulation is maintained in the donor compartment throughout the experiment the drug concentration remains more or less

constant for the length of the experiment. That is why these types of experiments are called as "infinite" dose experiments. They are also sometimes termed as "flux" studies[15,16,17]. Some researchers have tried to model the transport processes by considering the individual role of the skin layers e.g. stratum corneum, epidermis and dermis . Such approaches were used to mathematically predict the percutaneous permeation of betamethasone 17-valerate through human skin [18].

Retention is defined as drug residence in the skin layers or drug localization in the skin. This term is relevant to dermatological products when formulation is applied in a finite dose manner (one-three mg/cm^2). A comparable term is penetration. Retention can be expressed quantitatively as drug concentration in the skin e.g. $\mu g/gm$ (whole skin, epidermis or dermis) or as a percent of the dose applied [12]. One can carry out in vivo or in vitro experiments to optimize the dermatological formulations. In vivo studies are carried out by applying dermatological formulations on the abdominal region and excising the skin after a certain period of time. The time of the experiment may vary with different researchers. The animal is sacrificed at the end of the experiment and the skin is excised. Drug concentration can be expressed in the whole skin or in individual layers e.g. epidermis and dermis. The skin may be sectioned into

individual layers by using a microwave technique[19] or by microtome slicing of the skin[20]. Since only 1-3 mg of the formulation is applied per cm^2 these type of experiments are termed as the "finite" dose experiments [1,2,19-23].

Figure 3 shows a more composite schematic representation of the local events which take place upon the application of a transdermal or dermatological preparation on the skin surface[3]. This diagram efficiently depicts the fate of a drug when it is released/liberated from the applied dermatological or transdermal formulation.

Although using the human in vivo model would be the best choice for evaluating both transdermal and dermatological products it is impractical to use primarily because of the cost and other practical factors. However by using animal models which have the relevant skin characteristics similar to man can be successfully substituted for human studies for optimizing dermatological or transdermal formulations prior to clinical trials. As is explained in detail in the following sections a variety of animal models have been used.

1.3. Assessment of Skin Permeation and Retention

Over the decades, a large number of models, methods and membranes have been explored to assess drug delivery from

transdermal preparations. These models were designed to answer many questions including drug release from transdermal products, rates of drug transport across the skin, optimization of drug delivery by using

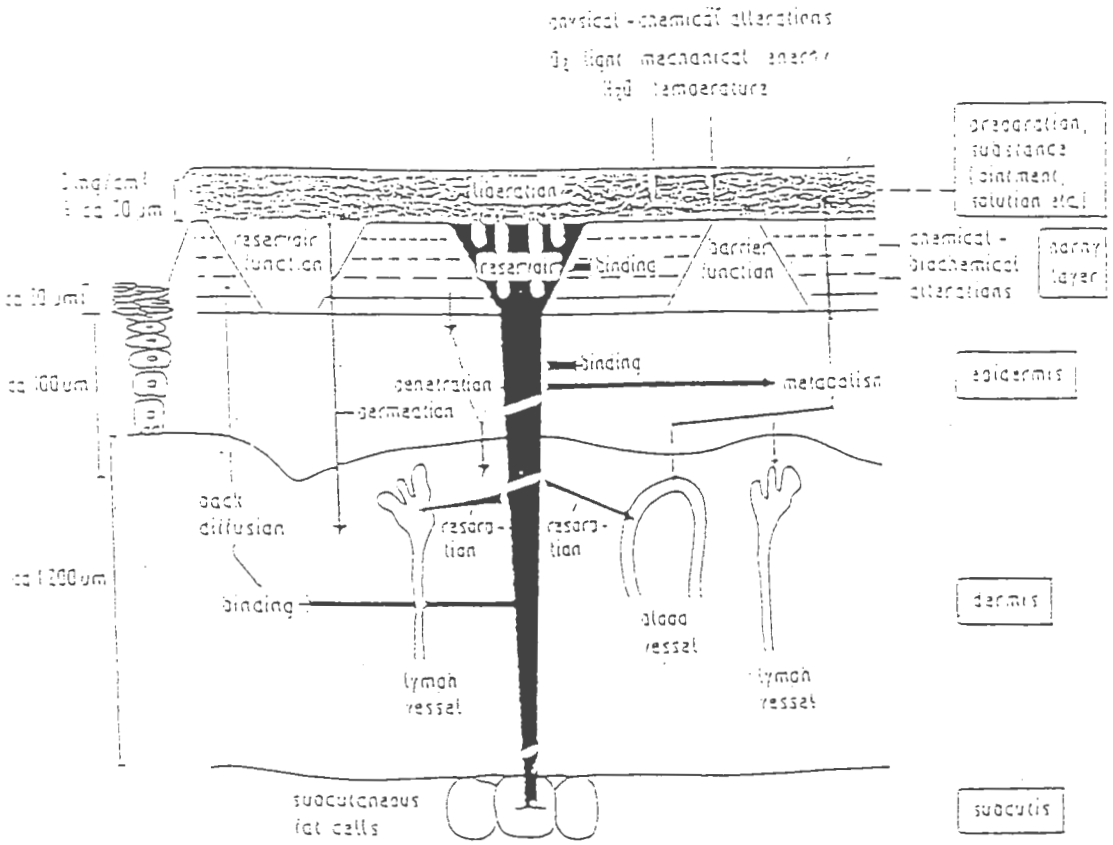


Figure 3. Schematic Representation of Local Events After Application of a Transdermal or Dermatological Formulation (Adapted from Shaefer, H., et al., 1982.[3])

enhancers, mechanisms of drug transport, study of various factors which affect drug delivery and use of prodrugs. Most studies reported in the literature are related to transdermal drugs.

Several types of animals have been explored as possible models for human skin. A rather interesting list compiled from these studies is provided in Table 1 [24].

As can be seen, the animals explored range from mouse to horse. Of course all of these vastly varying animal skins can not possibly be the right model for the human skin. The criteria for comparability used in the reported studies included total percent permeation, permeability coefficients, blood levels, urine levels, and pharmacological and pharmacodynamic responses. This list may appear to be extraordinarily long, but it is by no means complete. However, it does provide an indication of diversity in a researcher's choice of animal models which presumably mimic the permeability of human skin.

Table 1. List of Animal Models Used in Transdermal and Dermatological Drug Delivery[24].

#	Animals	In Vivo	In Vitro	References
1.	Hairless Mouse		X	25
2.	Swiss (Furry Mouse)		X	26
3.	Athymic Nude Mouse		X	24
4.	Furry Rat	X	X	27
5.	Fuzzy Rat	X	X	24
6.	Nude Rat	X	X	28
7.	Hairless Rat	X	X	29, 30
8.	Hairless Guinea Pig	X	X	31
9.	Guinea Pig	X	X	32
10.	Weanling Yorkshire Pig	X		33
11.	Yucatan Miniature Pig	X		34,35
12.	Mini Pig	X		27
13.	Micro Pig	X		24
14.	Domestic Pig	X		36
15.	Cat	X		37
16.	Dog	X		37
17.	Mexican Hairless Dog	X		38
18.	Hairless Dog	X		39
19.	Syrian Golden Hamster		X	40
20.	Sheep	X		41
21.	Chimpanzee	X	X	37
22.	Rhesus Monkey	X	X	42
23.	Squirrel Monkey	X		27
24.	Rabbit	X	X	30
25.	Goat	X		37
26.	Horse	X		37
27.	Snake (shed skin)		X	43,44

Skins from these models can be classified into the following three major categories:

a. Furry skins
b. Fuzzy skins
c. Hairless skins

Furry skins are not suitable models for human skin. Hairless skins provide the permeation and retention information which is mechanistically closer to the human skin. A review of literature indicates that different investigators have claimed that the permeability of a variety of skins resembles that of the human skin[24]. The models referred to here can be classified as follows[21]:

- Human In Vivo
- Animal In Vivo
- Cultured Skin Membranes
- Human In Vitro
- Animal In Vitro
- Synthetic Membranes

1.3.1 Human In Vivo: Estimation of a drug's permeation or retention using human in vivo model is, of course, most preferred. But, it is also the most difficult and non-practical model to use. Rarely would one get an opportunity to routinely use this model during the development of a transdermal or dermatological product. Drug delivery assessment is invariably made by using non-human models.

However, towards the completion of the development, one should estimate drug delivery in humans. Efforts should be made at every opportunity one gets to use the human *in vivo* model so that a historical data base can be built for the correlation of human data with those from animal or other studies.

1.3.2. *Human In Vitro*: A large number of studies have been reported on drug permeation through excised human skin, only a few will be referred here [41,45-51]. These studies were done by placing a piece of the skin in between two halves of a diffusion/permeation apparatus and by monitoring drug transport into the receptor compartment. The *in vitro* approach has been used by many researchers in assessing the feasibility of developing transdermal products. It is important to note that *in vitro* data may not always correlate with *in vivo* data. Recently, some investigators have used this approach in evaluating dermatological products. As was pointed out at the International Workshops on Dermatological Products[1,2] this approach does not necessarily provide a correct answer to the question of drug delivery to the skin layers. Drug retention and not drug permeation is the correct and relevant criterion in evaluating dermatological products. It has been shown that the kinetics of drug permeation and retention are not necessarily correlated [19,21-23]. Permeation and retention of drugs can be differently influenced for different drugs by formulation factors.

Some scientists have used human in vitro models to evaluate dermatological products by monitoring drug retention in the skin layers at the end of a permeation experiment. This approach, in concept, is fine but, due to differences between in vivo and in vitro studies, one may not get correct and meaningful data. These differences can arise due to the following reasons:

a. Inherent differences in the transport pathways. Certain transport pathways through the skin may behave differently in the in vitro vs in vivo situations. These pathways may include the follicles and sweat glands.

b. Receptor compartment fluid can migrate upward into the donor compartment and change the characteristics of the applied formulation thus altering the permeation and retention properties of the applied drugs.

c. The relative abilities of the epidermis and dermis to permeate and retain drugs can change due to the migration of receptor fluid into the skin layers.

d. Drugs need to cross the entire skin thickness in the in vitro system. However, in the in vivo system, they start getting picked up by the circulation just past the viable epidermis and do not have to traverse the entire skin

tissue. This can provide differing permeation/retention data.

e. The enhancing effects of most formulation excipients are likely to exert different effects in in vitro vs in vivo systems.

While there are good reasons to use excised human skin, there are disadvantages:

a. It is difficult to obtain human skin of a desirable age, gender, race, site and quality. Therefore, it is difficult and often not possible to keep these parameters constant in a given study.

b. Invariably, the skin needs to be stored prior to use. Different investigators use different storage conditions which are likely to affect results.

c. The skin has to be free from any contagious disease. This restricts the sources of skin supply.

d. The cost considerations.

1.3.3. Animal In Vivo: Animal in vivo studies are more desirable than in vitro studies due to their relevance.

However, such studies have not been extensively carried out and reported [28,29,52-55]. These studies were concerning both transdermal and dermatological drugs. For the latter drugs, the criterion of drug delivery was drug concentration in the stratum corneum determined by using the tape stripping and autoradiographic technics. Recently, another in vivo approach has been developed[56]. This approach used an in vivo hairless guinea pig model and provided blood level data for transdermal compounds. By comparing results with the pharmacokinetics in humans, if known, or by making appropriate assumptions, one can predict blood levels in humans. This model has already been used successfully.

Similarly, an in vivo model for dermatological drugs is preferred over an in vitro model. In this model, a formulation is applied in a finite dose manner and drug retention is determined in the epidermis and dermis. Various potential formulations can be evaluated and ranked meaningfully using such a model. A hairless guinea pig model has been developed and has been used extensively in screening dermatological formulations at Hoffmann-La Roche, Inc. for the past several years[56]. The disadvantages of in vivo animal models include the cost involved and need for animal facilities.

1.3.4. Animal In Vitro: This model has been extensively used in evaluating transdermal drugs. The model suffers from the

same disadvantages as those mentioned in the section, Human In Vitro. An additional concern is the morphological properties of the animal skins used in these studies. Most studies reported used furry skins. Mechanistic studies have shown that only "hairless" skins provide transport pathways comparable to the human skin[24]. Hairless skins are not fully devoid of hair follicles. The residual follicles and the overall skin structure provide transport pathways and effects of physicochemical parameters similar to those found in the human skin. Data obtained from "furry" skins is **irrelevant** and **non-representative** to human skin.

Almost all of the reported studies using animal in vitro models pertain to transdermal drugs[27,42,57-59]. The use of excised animal skins in evaluating dermatological formulations for drug retention in the skin layers is rather limited[19,20,22,23]. The results are greatly influenced by the artefact of using an in vitro system. Therefore, the use of in vitro animal models for dermatological drugs should be discouraged.

1.3.5. Cultured membranes: Recently, a lot of research has been done by Organogenesis Inc. and Advanced Tissue Sciences in producing cultured skin[60,61]. The cultured membranes were developed for grafting purposes. As additional applications, these membranes were also evaluated for their use in screening drug substances for pharmacological and toxicological

effects[62-66]. Success in these efforts led the companies to promote the cultured skins for their applications in assessing drug delivery[67]. A question was raised if these membranes can in fact provide drug permeation information which is relevant to the human situation. A mechanistic study carried out by using model compounds indicated that the transport characteristics of the cultured membrane were not comparable to those of the human skin[68]. The use of cultured skins in evaluating dermatological formulations is not a viable alternative. This is so due to the reasons given in an earlier section "Human In Vitro". As was stated earlier, in vitro models are not relevant to assessing local drug delivery.

1.3.6. Synthetic Membranes: The only value of using synthetic membranes, e.g. Silastic^{R}, cellulose acetate, polyurethane, etc. in the evaluation of transdermal and dermatological formulations is to understand/interpret data obtained from skin studies[21,69-77]. Such mechanistic studies have been carried out to differentiate physicochemical and biological factors, which affect drug transport. Synthetic membranes are not meant to provide predictive information on the actual drug transport into or through the skin. Therefore, these membranes have only a limited value in the development of transdermal or dermatological products.

1.4. Factors Which Affect Drug Delivery Into and Through the

Skin

An abundance of information exists in the literature on factors which affect drug delivery through the skin. Due to the emerging nature of transdermal products, many researchers have focused on the factors which affect drug flux across the skin. While these factors may not necessarily be relevant to drug delivery from dermatological products, they may provide some insight in as far as the barrier nature of the stratum corneum is concerned. The factors may be broadly divided into three categories viz., **physiological** including hydration, age, gender or site; **drug dependant** including drug lipophilicity and drug solubility and concentration; and **vehicle dependant effects**. These factors will be discussed further.

1.4.1. Physiological Factors: As mentioned earlier hydration or occlusion is probably the most notable of the factors studied[37,78]. It was long believed that hydration increased skin permeability of all drug substances[78-81]. However, this was shown not to be the case via permeation studies with test permeants, n-alkanols[25,26]. In the hairless mouse and furry mouse models, it was shown that hydration did not affect the permeation of polar compounds. The transport of moderately polar/non-polar compounds increased substantially with hydration. The permeation of non-polar compounds actually decreased with hydration. Virtually no studies have been

reported on the effects of hydration on drug delivery to the skin layers from dermatological products. It is an important piece of information missing because it would appear that effects of hydration are equally or more important for dermatological drugs. Many dermatological vehicles and other formulation excipients cause hydration of the skin. Age, gender and site have also been reported as important factors which can affect the permeation and retention of drugs[82]. These effects may be compound related. Christopher and Kligman in 1965, and Wildnauer and Kennedy in 1970[83,84] reported a lack of age-effects on the in vitro permeation of water in human skins. For fluorescein, however, a seven fold higher in vitro permeation was noted in older skins. In a study carried out using male and female hairless and furry mouse skins, and alkanols as test permeants, the age effects were studied over the entire age span of the animal[85-87]. It was found that the role of various pathways of drug permeation may change with the age of the skin. Notably, the follicular pathway was most affected in the first quarter of the age span. Gender is perhaps not a critical factor. Studies with alkanols in hairless and furry mice, and with propranolol in human skin, did not show substantial gender related changes[84,86,88].

The site of application on the body has a substantial effect on the drug permeation[42,85,86,89,90]. Large site effects were reported by these workers. The primary cause of these effects

is morphological differences in the rate controlling layer, stratum corneum. The effects of age, gender and site on the local drug delivery have not been reported.

1.4.2. Drug Dependant Factors: Drug lipophilicity can be expressed by partition coefficient and is probably the most important factor which affects drug permeation. Scheuplein and co-workers[91] studied this factor systematically with alkanols and found that permeation through the lipoidal pathways of human skin increased exponentially. A strong relationship was reported between drug permeation and octanol/water partition coefficients of several different drugs[77]. This partition dependence is due to the lipoidal nature of the physiological barriers. If this barrier layer is altered by either stripping of the cornified cells or other means, the effect of partition coefficient becomes less important[21]. Partitioning of drug in the stratum corneum is also affected by formulation factors such as choice of vehicles, the type of dosage form, and particle size of the drug. Among these factors the vehicle effects is a very important factor.

While majority of the studies reported pertain to drug permeation, some researchers have noticed a correlation between partition coefficients and therapeutic effects in topical drug delivery. For example, of 23 esters of betamethasone studied, the 17-valerate had the highest topical

activity which directly correlates with the therapeutic activity of the drug and the lipid-water partition coefficient[92].

Drug solubility and concentration are also well studied factors for transdermal drugs. While there is rarely an exception to the dependence of flux on drug concentration, same argument cannot be made for drug solubility. Theoretically, drug permeation from saturated solutions would be highest because of the maximum thermodynamic activity of the drug. However, data reported on several drugs demonstrated that saturation flux was not dependent on drug solubility[19,72,73,74]. This departure from the theoretical principles was attributed to the violation of following assumptions:

a. Only the drug molecule will permeate into and through the skin.

b. The skin will not be altered in any manner during the permeation process.

c. The microenvironment in the skin layers will not be altered by the presence of drug and formulation excipients.

d. Drug concentration and therefore thermodynamic activity always remains constant in the formulation applied on the skin.

The effects of drug solubility and concentration on drug retention are far less explored. No definite trend was observed between retention and solubility for two drugs, a PAF antagonist and a lipoxygenase inhibitor[22,23]. In these studies, effects of drug concentration on its retention in the epidermis and dermis were also examined. It was found that drug retention increased linearly with solubility in the epidermis while it increased asymptotically with solubility in the dermis.

1.4.3 Vehicle Effects: Vehicle effects on the drug permeation have been extensively studied[14,57,93-101] whereas only few reports exist on local drug delivery[19,20,22,23]. Among the ones that were previously talked about, the following three factors affect the extent and the rate of drug permeation through the skin: vehicle, skin and drug. The first factor includes vehicle/skin partitioning, drug solubility, occlusive nature of formulations, drug concentration and pH of the formulation. Skin related factors include the skin itself, integrity of the barrier layer, skin hydration, vehicle retention in the skin and condition of the skin prior to formulation application. Irrespective of these two factors, the drug uptake into the

skin is changed because vehicles alter the barrier function of the stratum corneum. The skin related factors are known to be more critical than physicochemical factors[97].

Vehicle effects were evaluated by drug dissolved in a vehicle[19,21-23,102,103] e.g. estradiol, PAF antagonist, lipoxygenase inhibitor, or mixed vehicle[50,93,94,96,104-106] e.g. fluocinolone acetonide, fluocinonide, indomethacin, adenosine etc., or more sophisticated formulations, e.g. emulsions[107], gels[108-110], e.g. idoxuridine, tretinoin, etc, or ointments[52,111] e.g. betamethasone derivatives etc., liposomal preparations[109,112], e.g. tretinoin, or niosomal preparations[113,114], microsponge systems[115], or polymeric microspheres[116]. Most of these effects were studied in vitro and both lipophilic and hydrophilic drugs were used. The effects on drug delivery exerted by different vehicles and dosage forms varied a lot.

One effect has been the topic of much research and interest, effect of vehicles on the state of hydration of the skin[117]. In contrast to the above effect, vehicles which are immiscible with water and are lipoidal in nature, show an opposite effect on water loss from the skin and, therefore, on the state of hydration of the skin. Such vehicles reduce both insensible perspiration and the release of sweat. The sweat collects at the openings of the glands but, does not spread as a film between hydrophobic skin surface and hydrophobic vehicle

because the free surface energy of the vehicle-skin surface is smaller than that between water and the skin[107]. For example if a lipophilic layer of a vehicle is present, it is not spontaneously replaced by the water-skin boundary layer if sweat is secreted[105]. Lipophilic waxes and single phase vehicles such as isopropyl myristate possess approximately equal moisture occlusive properties in their resistance to moisture loss as does Saran Wrap Film[105,118-121].

Mollgaard and Hoelgaard in 1983[97] conducted a thorough study to investigate the effects of 21 vehicles on the in vitro flux of estradiol from saturated solutions through excised human skin. These single vehicles ranged from commonly used vehicles, e.g. propylene glycol, PEG 400, glycerol to experimental vehicles, e.g. ethylene glycol, diethylene glycol, hexanetriol, decanol, etc, to controversial vehicles, e.g. dimethylsulfoxide. The flux results as shown in Table 2, showed a 220 fold range, from 0.001 $\mu g/hr/cm^2$ to 0.22 $\mu g/hr/cm^2$. Lag times for the steady-state permeation were also provided. They ranged from zero hour for DMSO to 57 hours for triethylene glycol and triethanolamine.

Table 2. Ranking of Different Vehicles According to the Flux of Estradiol[97]

#	Vehicle	Flux ($\mu g*cm^{-2}*h^{-1}$)	Lag Time (hrs)	Ranking
1.	Ethylene Glycol	0.22	13	1
2.	Propylene Glycol	0.12	18	2
3.	Dimethyl Sulfoxide	0.14	0	3
4.	Diethylene Glycol	0.12	53	4
5.	Propanediol(1,3)	0.11	39	5
6.	Triethylene Glycol	0.087	57	6
7.	Dipropylene Glycol	0.047	54	7
8.	Glycerol	0.037	26	8
9.	Betahistine	0.028	8	9
10.	Methyl Salicylate	0.027	11	10
11.	Ethyl Oleate	0.025	14	11
12.	Diisoamylamine	0.019	12	12
13.	2-amino-4-methylhexane	0.018	11	13
14.	Butanediol(1,4)	0.017	43	14
15.	Decanol(1)	0.014	18	15
16.	Triethanolamine	0.010	57	16
17.	Hexanetriol(1,2,6)	0.008	28	17
18.	Diethanolamine	0.007	5	18
19.	Pentanediol(1,5)	0.005	28	19
20.	PEG 400	0.001	-	20
21.	Tween 80	0.001	-	21

Transdermal and dermatological formulations typically contain more than one excipient. However, it is still useful to study skin uptake from single vehicles because it allows to choose appropriate vehicles for the final formulation. Some of the marketed dermatological products in fact contain large amounts of liquid vehicles. Examples include Retin-A^{R} Lotion, Synalar^{R} Lotion and Topicort^{R} Lotion. For many of these products, the consistency (viscosity) is close to that of "neat" vehicles. A list of marketed products has been provided for the reader to get an idea of the type of vehicles typically used in dermatological formulations (see Table 3).

DMSO is probably the most controversial and most studied vehicle in the transdermal and dermatological fields[123-127]. Soon after its use in therapeutic applications became known in mid 1960s, DMSO became popular and widely used. However, an alarming report was issued by Huntington Research Center in England that DMSO can change the refractive index of the lens in the eyes of rabbits, pigs, and dogs. At that point, the FDA prohibited the therapeutic use of DMSO despite the fact that the new Huntington results contradicted its earlier conclusions as to the safety of DMSO. Extensive safety studies followed and it was concluded that no one had actually observed a single case of damage to the human eye due to DMSO[128] and that DMSO is a very safe compound for human use[129].

Table 3. List of Some of the Most Commonly Used Marketed Dermatological Products[122].

#	Drug, Strength	Product Name (Manufacturer)	Vehicle(s)*
Indication : Abradant			
1.	Urea	Ureacin Lotion (Pedinol)	PG, MO
Indication : Acne			
1.	Erythromycin, 2%	A/T/S Topical Solution(Hoechst)	PG, Alcohol (66%)
2.	Benzoyl Peroxide, 2½, 5 and 10 %	Benzac AC Water-Base Gel (Galderma)	Alcohol (12%)
3.	Erythromycin (3%), Benzoyl Peroxide (5%)	Benzamycin Topical Gel (Dermik Labs)	Water, Alcohol (16%)
4.	Benzoyl Peroxide, 4%	Brevoxyl Cleaning Lotion (Stiefel Labs)	Water, PG
5.	Clindamycin, 2%	Cleocin T Topical Gel and Solution (Upjohn)	Gel: PEG 400, PG, water Solution: IPA (50 %v/v), PG, water
6.	Tretinoin,	Retin-A Liquid (Ortho-MacNeill)	PG, Alcohol, water
7.	Erythromycin, 2%	Theramycin Z Topical Solution (Medicis)	PG, Alcohol (81% v/v)

cont'd...

Table 3 (Cont'd)

#	Drug, Strength	Product Name (Manufacturer)	Vehicle(s)
Indication: Antibacterial			
1.	Benzoyl Peroxide, 2, 5, 10 %	Desquam-X Gel, 2,5, 10 (Westwood-Squibb)	Water, Carbomer 940
2.	Erythromycin, 2%	T-Stat 2 % Topical Solution (Westwood-Squibb)	Alcohol (71.2%), PG
Indication : Antibiotics			
1.	Clindamycin, 2%	Cleocin T Topical Gel and Solution (Upjohn)	Gel: PEG400, PG, water Solution: IPA (50 %v/v), PG
2.	Neomycin Sulfate, Polymyxin B Sulfate and Hydrocortisone 1%	LazerSporin-C Solution (Pedinol)	Water, PG
Indication : Antiinflammatory Agents			
1.	Podofilox, 0.5 %	Condylox Topical Solution (Oclasson)	Alcohol (95%)
2.	Amcinonide, 0.1%	Cyclocort Topical Lotion (Fujisawa)	Glycerin, IPM, PEG 400
3.	Betamethasone Dipropionate, 0.05%	Diprolene Lotion (Schering)	Water, IPM, PG

Cont'd...

Table 3 (Cont'd)

#	Drug, Strength	Product Name (Manufacturer)	Vehicle(s)
Indication : Dermatitis Relief			
1.	Desonide, 0.05%	Desowen Lotion (Galderma)	MO, PG
2.	Hydrocortisone Butyrate, 0.1 %	Locoid Topical Solution (Ferndale)	IPA (50%), Glycerin
3.	Hydrocortisone , 1, 21/2 %	Hytone Lotion (Dermik Labs)	PG, water
Indication : Pruritis			
1.	Amcinonide, 0.1%	Cyclocort Topical Lotion (Fujisawa)	Glycerin, IPM, PEG 400, water
2.	Floucinolone Acetonide, 0.1%	Synalar Topical Solution (Syntex)	PG,water
3.	Clobetasol Propionate, 0.05%	Temovate Scalp Application (Glaxo)	IPA (39.3%), water
Indication : Psoriasis			
1.	Betamethasone Dipropionate, 0.05%	Diprolene Lotion (Schering)	Water, IPM, PG
Indication : Hair Growth			
1.	Minoxidil, 2%	Rogaine, For Topical Use (Upjohn)	PG, Alcohol, water

*PG = Propylene Glycol
IPM = Isopropyl Myristate
IPA = Isopropanol
MO = Mineral Oil

Comment: Note that the products may contain additional excipients. Only those excipients mentioned in the PDR are reported here.

The effect of DMSO on the skin uptake of drugs is dependent on its concentration in the vehicle. Small increases were observed upto
50 % concentration in water. Above 50 %, the effect increases exponentially[130-135].

Propylene glycol and ethanol are also extensively studied vehicles[52,79,93,95,117,136-142]. It is noteworthy to mention these two vehicles together because they have been used in several marketed products including Retin-A^{R} Lotion, Ureacin Lotion, Cleocin-T lotion and Rogaine^{R} Topical Solution. The combination vehicle was used in these products apparently because it provided an optimal drug efficacy. It is not clear if this combination vehicle provided an optimal topical drug delivery (retention) too.

Additional studies have appeared in the literature on the permeation enhancing effects of these vehicles when used separately. Turi in 1979[143] showed that the permeation of diflurasone diacetate was enhanced by propylene glycol. The enhancement was observed to be proportional to propylene glycol concentration in the formulation. Propylene glycol was found to be effective in increasing the flux of fluorouracil[144] and hydrocortisone[145].

The enhancing effects of ethanol on drug permeation have also

been extensively studied[146]. At least two transdermal products on the market, Estraderm[R] [122,147-149] and Duragesic[R] [150] contain ethanol as a major component. From mechanistic standpoint, ethanol is probably the most investigated vehicle for its effects on drug permeation. Many researchers generally believe that the increased skin permeability results from denaturation or conformational changes in the keratin of the stratum corneum[151-154]. Under appropriate conditions, some hydrogen-bonding solvents, e.g. DMSO, ethanol, DMF, methanol, and propanol can open-up the stratum corneum cells and thus increase the diffusivity of many compounds[135,154-157]. Some investigators believe that "increased lipid-phase fluidity" is related to the increased permeability of the skin [156-161]. These researchers confirmed their speculations and beliefs by conducting differential Scanning Calorimetric, Infrared Spectrometry and Fourier Transform Infrared experiments.

It has been suggested that alcohol enhances drug partition into the stratum corneum which then results in enhanced drug permeation[146,162-166]. These studies were conducted on several compounds which included nicardipine. nitroglycerin, estradiol and an unidentifiable ionizable compound. Some common observations were made on these reports by Yum, et al. in 1994[146] and are interpreted as follows:

a. Drug solubility appeared to be an exponential function of

ethanol concentration in the vehicle. This point ,however, may not apply to all drugs and may very well be a function of the composition of the vehicle.

b. Transdermal flux of the drugs tested appeared to be an exponential function of ethanol concentration. Again, this relationship may not hold true for all drugs. The other components in the vehicle as well as the inherent properties of the drug itself can greatly influence the flux vs ethanol concentration.

c. The enhanced flux across the epidermis or stratum corneum appeared to be directly proportional to drug concentration in the tissue which in turn increased with increasing ethanol concentration. This observation should be taken with a word of caution. Flux experiments for transdermal drugs are normally done under infinite dose conditions and the conclusions may not necessarily apply to the finite dose situations which prevail for dermatological drugs. Besides, if the drug concentration in the tissue is determined only at the end of a flux experiment, the observed relationship pertains only to steady-state transport situation.

Azone^{R} (1-dodecylazacycloheptan-2-one) is another extensively studied vehicle, although it is better known as an enhancer[31,167-173]. Numerous reports have appeared in the

literature on the effects of Azone[R] on the permeation of various compounds[174-182]. Many of these drugs are listed in Table 4. Both types of studies, screening of drugs for assessing transdermal feasibility as well as mechanistic aspects have been carried out. It was found that solute delivery typically increased with Azone concentration[31] contradicting some researchers belief that enhancement of permeation is a saturable process with a maximal enhancement associated with a particular optimal concentration of Azone [167,174]. Vehicle selection plays an important role[31] in:

a. Whether or not the enhancement effects will plateau.

b. Producing a maximal Azone[R] concentration beyond which permeation will actually decline.

In spite of the extensive evaluation of Azone, it is not apparent that it will find its place in any marketed product. It is so because it does not provide any unique properties with regard to drug delivery.

Acetone has been used by many researchers to evaluate transdermal and dermatological drugs[183,184]. Guinea pig was used as an animal model for human skin absorption of hydrocortisone, testosterone and benzoic acid by studying permeation from acetone solutions[185]. The enhancing effect of

acetone on the permeation of water through murine skin was compared with that of DMSO, chloroform, ethanol and ether[186]. The enhancing effects of these four vehicles were found to follow the following rank order: Chloroform > ether > DMSO > acetone > ethanol. Several other compounds have been studied by using acetone as a vehicle[155,187]. According to them, acetone readily evaporates from the skin surface leaving behind a "solid penetrant" from which drug permeation or retention occurs. Many laboratories routinely utilize acetone as a vehicle of choice to evaluate new drug compounds for pharmacological effects in in vitro and in vivo models. Whether or not acetone is a suitable vehicle for such purposes is a topic for on-going debate[1,2].

Isopropyl myristate has been found to be an effective vehicle for many drugs. The permeation of fluocinolone acetonide and its acetate through human skin increased when isopropyl myristate was used in combination with volatile vehicle, isopropanol[104]. Smaller increases were noted when the volatile vehicle component was not allowed to evaporate, i.e. when the site of application was occluded. The permeation of acyclovir through hairless mouse skin was studied using vehicles including isopropyl myristate[57]. In this case the enhancer, 1-farnesylazacycloheptan-2-one (7 FU), 1-geranylazacyclovir-2-one (7 GU), 1-geranylazacyclopentan-2-one (5 GU) and Azone, were not found to be effective when used in isopropyl

myristate vehicle. It was reasoned that the enhancers and the vehicle were too similar to each other with respect to the physico-chemical properties. Pretreatment of the skin with some vehicles including isopropyl myristate, increased the permeability of the skin for compounds studied later[158]. It is likely that the pretreatment allowed the vehicle to permeate into the skin and be

Table 4. Drugs That Exhibit Azone-Induced Permeation Enhancement[31,181].

#	Class	Compound
1.	Antibiotics	Clindamycin Phosphate Erythromycin base Erythromycin
2.	Antifungals	Metronidazole Griseofulvin
3.	Antivirals	Cytarabin Idoxuridine
4.	Anthelmintics	Thiabendazole
5.	Antimetabolites	5-Fluorouracil
6.	Depigmenting Agents	Hydroquinone
7.	Corticosteroids	Amcinonide Triamcinolone Acetonide
8.	Nonsteroidal Antiinflammatory Drugs	Ibuprofen Indomethacin
9.	Narcotic Antagonist	Naloxone
10.	Cardiovasculars	Amlodipine Isosorbide Dinitrate
11.	Alkaloids	Morphine
12.	Sympathomimetics	Isoproterenol
13.	Benzodiazepines	Midazolam Maleate Diazepam

retained there. The drugs soluble in the vehicle component retained in the skin will have a better chance to be transported across the skin. With time, the vehicles themselves will be getting into the skin and therefore creating and maintaining a new microenvironment which is more favorable to these drugs studied. Washitake, M, et al.[189] investigated the in vitro permeation and retention of salicylic acid and carbinoxamine from four oily vehicles including isopropyl myristate. The vehicles which had better affinity for the drug, provided a lower skin permeation. Using neat vehicles, the enhancing effects of four vehicles on the limiting flux (flux from saturated solutions) through hairless mouse skin ranked: isopropyl myristate > propylene glycol > medium chain triglyceride > polyethylene glycol 400 > water > mineral oil for Ro 10-1670[72]. The rank order for Ro 15-1570 was: medium chain triglyceride > isopropyl myristate > polyethylene glycol 400 > mineral oil[73]. For another compound, isotretinoic acid in excised human skin, the rank order of the vehicles tested was isopropyl myristate > medium chain triglyceride > polyethylene glycol 400 > ethanol/water/(polyethylene glycol 400) mixture[74]. For the three drugs tested, no definite relationship between the limiting flux and drug solubility was observed. These studies only stress the fact that vehicle effects on drug permeation/retention are complicated and for most part are dependent upon the specific drugs used.

Polyethylene glycol 400 is also a commonly used vehicle in dermatological formulations. Generally, this vehicle is regarded as an inefficient vehicle to provide optimal permeation/retention of drugs when used by itself[22,23,73]. Permeation of four retinoids was studied from five vehicles using excised monkey skin[82]. The results indicated that the permeation followed rank order: propylene glycol = isopropyl alcohol > mineral oil > diisopropyl adipate > polyethylene glycol 400. PEG 400 was found to be at the bottom of the vehicles studied for the permeation of Ro 23-3544 through excised skin of hairless guinea pigs[19].

Most of the vehicle effects mentioned are on the transdermal drugs. The effects of vehicles on topical drug delivery to the skin layers are not well studied and understood. Some of the recent studies, using both in vitro and in vivo animal skin models, have shown that vehicle effects on the local drug delivery are also dramatic and depend on the drugs used. The in vitro retention of Ro 23-3544 in hairless guinea pig skin was noted to vary by orders of magnitude for ten vehicles studied[19]. Vehicles ranked differently for the permeation data than for the retention data. Large vehicle effects were also observed on the in vivo epidermal/dermal retention of Ro 24-5413[22] and Ro 24-0238[23] from many vehicles studied.

Vehicle effects on topical drug delivery have also been

studied using the tape stripping technique. Influence of 17 simple vehicles including combinations of ethanol, water, ethylene glycol and triton X-100 were studied using hairless rat. The stratum corneum reservoir was assessed. It was found that the total amount of the drug that penetrated over four days varied by a factor of 50, thus demonstrating the importance of vehicle in skin absorption[29,54,55]. Pershing[190] investigated whether the stripping method could also predict the pharmacological effect of a drug. They showed that the total amount of betamethasone dipropionate in the tape stripped from human skin correlated with the vasoconstriction in vivo. A new penetration enhancer, HPE-101 increased the permeation of indomethacin through tape stripped guinea pig skin[191]. Some researchers have studied the retention of drugs in the skin after carrying out a flux study. The topical penetration of piroxicam was measured after an in vitro flux study through pig skin. It was concluded that the topical penetration of the drug is dependent on the distribution of local cutaneous vasculature[192].

Vehicle effects on drug retention are actually critically important for dermatological drugs but, unfortunately not much work has been done in this area. The awareness, however, has increased in the recent few years and it is hoped that a lot more will be learnt about local drug delivery and its optimization via a judicial selection of vehicles.

2.0 OBJECTIVES

The objectives of the present study were as follows:

1. To determine solubility of two model drugs, hydrocortisone (HC) and triamcinolone acetonide (TAC), in a wide variety of vehicles which are either used in topical drug delivery systems or are potential vehicles.

2. To study the effects of these vehicles on the *in vivo* retention of HC and TAC in the epidermis and dermis of hairless rat.

3. To draw interpretations from the observed data with respect to vehicle effects on the *in vivo* drug retention, drug retention *vs* solubility, and drug retention *vs* lipophilicity.

4. Since corticosteroids are poorly absorbed in the skin the results of this study are expected to aid in optimizing the selection of vehicles for topical drug delivery and in the better selection of vehicles for these two corticosteroids.

5. To further explore the *in vivo* hairless rodent model in screening potential dermatological formulations for local drug delivery as a step prior to clinical studies.

3.0. EXPERIMENTAL

3.1. Materials

3.1.1. Animals:

Male Hairless Rat (Strain: CD Hairless) was used as the animal model and animals of 8-12 weeks age were obtained from Charles Rivers Laboratories, Massachusetts. The animals were housed in a suitable facility and the environmental conditions were controlled. The animals had access to food and water ad lib.

3.1.2. Model Drugs:

Two glucocorticoids, hydrocortisone (HC) and triamcinolone acetonide (TAC) were chosen as model drugs. These two drugs have substantially varying ether/water partition coefficients, 1.3 for HC and 13.1 for TAC. The reported water solubility for hydrocortisone is 0.28 mg/mL and that for TAC is less than 0.1 mg/mL. The formulations of these drugs were "spiked" with their ^{3}H-labeled forms. The labelled and unlabelled drug compounds were obtained from the following sources:

Hydrocortisone	Sigma Chem. Co., St. Louis, MO.
Triamcinolone acetonide	Sigma Chem. Co., St. Louis, MO.
Hydrocortisone^{3}H	ICN Biomedical Co., Irvine, CA.
Triamcinolone acetonide^{3}H	ICN Biomedical Co.,

Irvine, CA.

3.1.3. Chemicals and Materials:The chemicals and materials used and
their sources are as follows:

Acetonitrile, HPLC Grade	Fisher Sci. Co., Fair Lawn, NJ.
Acetone, HPLC Grade	Fisher Sci. Co., Fair Lawn, NJ.
Water, HPLC Grade	Hoffmann-La Roche Inc., Nutley, NJ.
Tritium Cocktail	R. J. Harvey Inst., Co., Paramus, NJ.
Sodium Pentobarbital	Sigma Chem. Co.,St. Louis, MO.
Xylazine	Mobay Corp., New York, NY.
Ketamine	Fort Dodge Labs Inc.,Fort Dodge, IO.
Oxygen	JWS Technologies, Piscataway, NJ.
Nitrogen	Liquid Carbonic Inc.,Oak Brook, IL.
Carbon Dioxide	Liquid Carbonic Inc.,Oak Brook, IL.
Alcohol Swabs	Becton Dickinson, Inc.,Franklin Lakes, NJ.

3.1.4. Vehicles: The vehicles that were studied in this project are listed below along with their sources.

Ethylene Glycol	Dow Corning Co., Midland, MI.
Diethylene Glycol	Dow Corning Co., Midland, MI.
Propylene glycol	Sigma Chem. Co., St. Louis, MO.
PEG 200	BASF Chem., Washington, NJ.
PEG 300	BASF Chem., Washington, NJ.
PEG 400	BASF Chem., Washington, NJ.
Ethanol	Sigma Chem. Co., St. Louis, MO.
Isopropanol	Fisher Sci. Co.,Fair Lawn, NJ.
Isostearyl Alcohol	Fisher Sci. Co.,Fair Lawn, NJ.
Isopropyl Myristate	Unichema Chem., Chicago, IL.
Isopropyl Palmitate	Unichema Chem., Chicago, IL.
Miglyol 812^{R}	Huls Amer. Inc.,Piscataway, NJ.
Miglyol 840^{R}	Huls Amer. Inc.,Piscataway, NJ.
Imwitor 408^{R}	Huls Amer. Inc.,Piscataway, NJ.
Imwitor 412^{R}	Huls Amer. Inc.,Piscataway, NJ.
Dimethyl Sulfoxide	Sigma Chem. Co., St. Louis, MO.
Azone^{R}	Nelson Research, Irvine, CA.
Transcutol^{R}	Gattefosse, Inc.,St.Priest, France
Linoleic Acid	Sigma Chem. Co., St. Louis, MO.
Oleic Acid	Fisher Sci. Co.,Fair Lawn, NJ.
Monoacetin	Unichema Chem.,Chicago, IL.
Diacetin	Unichema Chem.,Chicago, IL.
Triacetin	Unichema Chem.,Chicago, IL.

Tributyrin	Sigma Chem. Co.,St. Louis, MO.
Tricaprylin	Sigma Chem. Co.,St. Louis, MO.
Triolein	Sigma Chem. Co.,St. Louis, MO.

3.2. Methods

3.2.1. Radioisotopic Analysis: Liquid Scintillation Counter LSC 9800, Beckman Instruments Co., and a specialized Tritium Cocktail, R. J. Harvey Instruments Co., Paramus, NJ, were used for the analysis of ^{3}H- labeled HC and TAC.

3.2.2. Determination of Solubility of HC and TAC in Various Vehicles: Solubility of HC and TAC was measured in the vehicles listed in 3.1.4. An excess amount of the drug was suspended in a measured amount of vehicle. The suspension was shaken at 37°C for 72 hours and was filtered into a preheated vial (37°C) by using a 5 mL syringe and a 0.45 μ filter which were also preheated at 37°C. The filtrate was diluted and assayed by HPLC as reported by Tymes, N. J., in 1977[193]. This method was capable of analyzing both, HC and TAC. The details of the HPLC instrument and experimental conditions used are provided in Table 5.

Standard Solutions were prepared by dissolving 15 mg of HC in 15 mL of acetonitrile and 15 mg of TAC in 15 mL of acetone to obtain a concentration of 1 mg/mL. The Precision of injections was checked by making four 10 μL injections of the Standard Solutions of HC and TAC. The percent relative standard

deviation of the area under the curve for both the drugs was below 2 % indicating satisfactory precision.

Table 5. Instrumentation and Experimental Conditions For HPLC Analysis of Hydrocortisone and Triamcinolone Acetonide.

#	Experimental Conditions	Description
1.	Pump	Waters Model 600E, Multisolvent Delivery System
2.	Injector	Waters WISP 712B Auto Sampler Injector
3.	Detector	ABI Programmable Wavelength Detector
4.	Column	Microbondapak C_{18}, 30 cm, 4 mm i.d.
5.	Mobile Phase	Acetonitrile:Water (40:60)
6.	Flow Rate	1.5 mL/min
7.	Injection Volume	10 μL
8.	Retention Time	Hydrocortisone $\cong$ 4.0 min Triamcinolone Acetonide $\cong$ 6.0 min
9.	Run Time	10 Minutes

The linearity of detection of HC and TAC was determined by serially diluting the Standard Solution (1 mg/mL) with acetonitrile for HC and with acetone for TAC. The dilutions were performed to obtain a concentration range of 1 μg/mL to 1 mg/mL. Plots of peak areas versus concentrations (Figures 4 and 5) showed linear responses with correlation coefficients over 0.999.

3.2.3. In Vivo Retention Procedures: The following experimental procedures were used to determine the in vivo retention of HC and TAC:

a. Preparation of Formulations: ^{3}H labelled HC and TAC solutions (1μCi/μL) were received from ICN Biomedicals, Irvine, CA, in 200 proof ethanol. The specific activity of HC was 56 Ci/mM and that of TAC was 49.5 Ci/mM. Fifty μL of the solution was diluted to one mL using 200 proof ethanol to obtain a concentration of 0.05 μCi/μL. Fifty four μL of this stock solution was pipetted in a vial and alcohol was evaporated under a constant flow of nitrogen to get an eventual count of about 30,000 dpm/μL ("Hot" Residue). Calculations indicated that this level of radioactivity was sufficient for radioisotopic analysis.

"Cold" solution of each drug was prepared in each vehicle at half of drug solubility to maintain a constant

thermodynamic activity[22,23,97]. Two hundred μL of this solution was added to the "hot" Residue. This mixture was sonicated for five minutes to ensure a uniform mixing of the "hot" and the "cold" forms of the drugs. The uniformity of mixing was checked by counting the radioactivity of three five μL aliquots of the mixture.

b. Preparation of Animals: Male hairless rats of age 8-12 weeks were preanesthetized by using a mixture of oxygen and carbon dioxide. The rats were anesthetized by injecting a mixture of ketamine (100 mg/Kg) and xylazine (10 mg/Kg) subcutaneously . Additional injections were made if needed. The abdominal region of the animals was washed with water to remove debris and a circular 10 cm^2 area was marked. The animals were laid on a heating pad against their backs.

c. Formulation Application: Twenty μL of the drug formulation was applied on the 10 cm^2 marked area (2 $\mu L/cm^2$) on the abdomen of the animal by using a glass rod. The formulation was gently spread and rubbed on the skin. Experiments were carried out under ambient conditions.

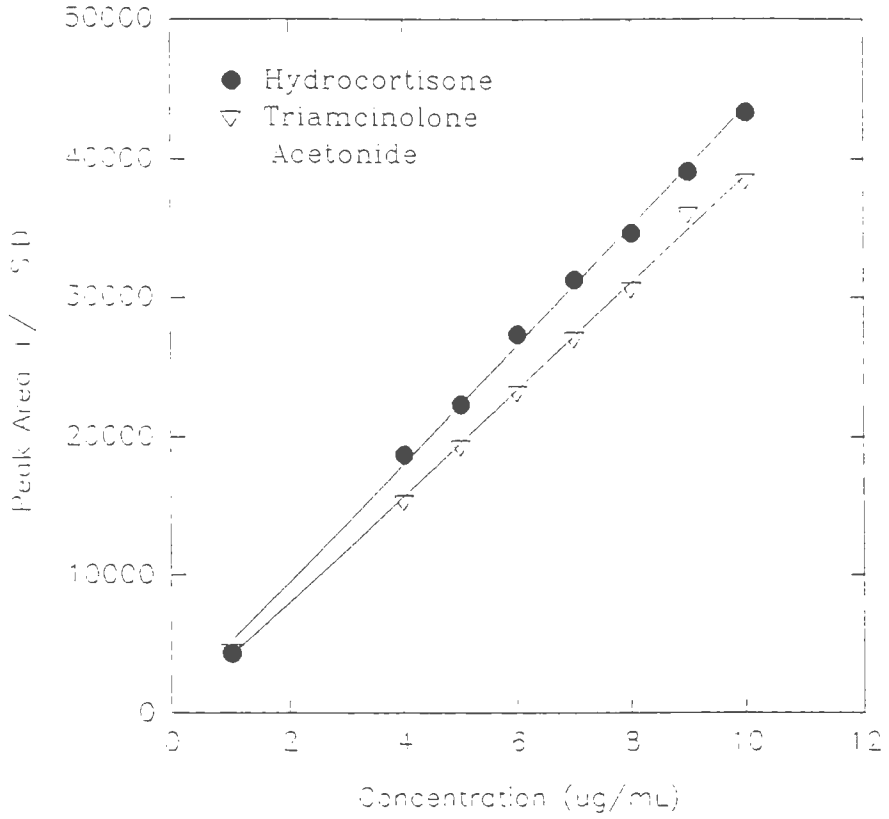


Figure 4. Linearity Plots of Peak Area vs Concentration for Hydrocortisone and Triamcinolone Acetonide in the range of 1 ug/mL to 10 ug/mL.

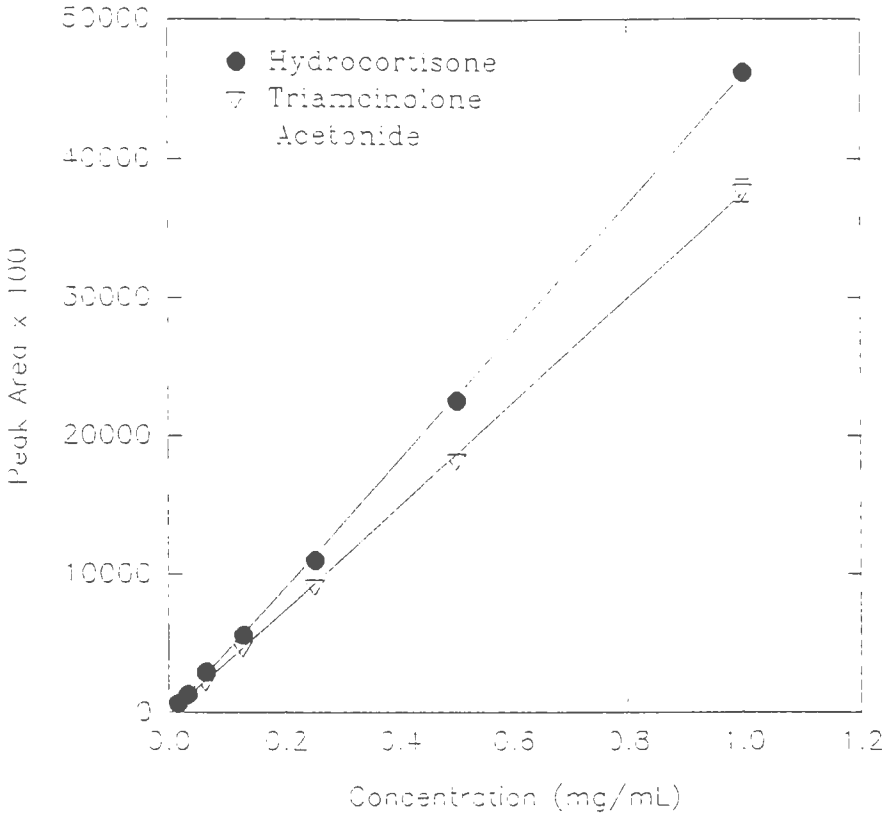


Figure 5.. Linearity Plot of Peak Area vs Concentration for Hydrocortisone and Triamcinolone Acetonide in the Range of 1 ug/mL to 1 mg/mL.

d. Excision of Skin: At the end of the experiment i.e. three hours, the animals were sacrificed by an intracardiac injection of pentobarbital (120 mg/mL), Sigma Chemical Co., St. Louis, MO. Excess formulation was removed by carrying out a "topwash" procedure. This was done by wiping the skin four times with each of six alcohol swabs used. The marked area of the skin was excised using a #21 scalpel and a forcep, and was placed in a petridish.

e. Sectioning of Skin Layers: The petridish containing the skin was microwaved for four seconds and the skin was sectioned into epidermis and dermis[194]. The epidermal and dermal layers were weighed and placed in scintillation vials.

f. Measurement of the Radioactivity in Skin Samples: The radioactivity was measured by oxidizing the skin layers in a RX-500 Biological Oxidizer, R. J. Harvey Instruments, Paramus, NJ. The oxidizer consisted of a combustion chamber which was under a constant flow of nitrogen and was maintained at 900°C. During the combustion, nitrogen was replaced with oxygen. The combustion was completed within four minutes. The skin layers were oxidized and tritium was captured as tritiated water in the scintillation fluid. The

scintillation vials were placed in an LSC 9800 and the radioactivity of the oxidized skin layers was determined.

3.3. Data Analysis: The observed radioactivity was converted into drug concentration in terms of drug amount by using the specific activity of the formulation. Drug concentration in skin layers was calculated by dividing the amount observed by the weight of the tissue. This was expressed in terms of μg/Gm. The data was also expressed as percent of the applied dose retained in the skin layer and was calculated as follows:

$$\% R = \frac{D}{Dose} \times Wt. \times 100$$

where %R = Percent of dose retained
D = Drug in μg/gm
D1= Dose Applied
Wt= Tissue weight in gm

4.0. RESULTS AND DISCUSSION:

4.1. High Performance Liquid Chromatography (HPLC) Method

An HPLC method was used to determine the solubility of HC and TAC. This method has been reported in the literature (Table 5) for the analysis of several corticosteroids. A lack of interference by 26 vehicles used in the present study with the method was established.

4.2. Solubility of HC and TAC in Vehicles Studied

Table 6 contains data on the solubility of HC and TAC in the vehicles tested. The vehicles belonged to the class of glycols, polyethylene glycols, alcohols, esters, glycerides, mixed commercial vehicles and conventional enhancers.

Generally, the trends in the solubility within each class of vehicles was similar for the two drugs tested. The solubility values for a given drug in a given class of vehicles, show substantial ranges. Overall, the solubility of HC ranged from 0.15 mg/mL to 286.8 mg/mL, a **1912 fold** range for all vehicles tested. These values for TAC ranged from 0.14 mg/mL to 321.7 mg/mL, a range of **2297 fold**.

Table 6. Solubility of Hydrocortisone (HC) and Triamcinolone Acetonide (TAC) in the Vehicles Tested.

#	VEHICLES	SOLUBILITY (mg/mL)	
		HC	TAC
1.	Ethylene Glycol	12.4	4.6
2.	Diethylene Glycol	25.2	15.9
3.	Propylene Glycol	12.0	12.1
4.	PEG 200	23.9	17.8
5.	PEG 300	17.3	15.5
6.	PEG 400	12.8	13.1
7.	Ethanol	10.1	23.2
8.	Isopropanol	4.46	3.40
9.	Isostearyl Alcohol	1.14	1.20
10.	Isopropyl Myristate	0.320	0.190
11.	Isopropyl Palmitate	0.180	0.140
12.	Monoacetin	12.2	16.7
13.	Diacetin	17.3	15.1
14.	Triacetin	4.00	3.80
15.	Tributyrin	0.250	0.190
16.	Triolein	0.230	0.220
17.	Tricaprylin	2.14	0.610
18.	Imwitor 408^{R}	8.30	7.90
19.	Imwitor 412^{R}	22.3	1.90
20.	Miglyol 812^{R}	0.420	0.490
21.	Miglyol 840^{R}	0.900	0.700
22.	Oleic Acid	0.150	0.190
23.	Linoleic Acid	12.2	0.27
24.	Transcutol^{R}	23.8	13.3
25.	Dimethyl Sulfoxide	286.8	321.7
26.	Azone^{R}	46.4	46.7

4.3. In Vivo Retention Studies

4.3.1. Effects of Vehicles: Details of the epidermal and dermal retention values for all the vehicles are given in the appendix A and B. Tables A.1. - A.26. and B.1. - B.26. contain data on the *in vivo* retention of HC and TAC for all the vehicles studied, respectively. Each table contains actual data obtained for the epidermis and dermis. Three animals were used for each experiment. Average retention values along with the respective standard deviations are tabulated.

Summaries of the *in vivo* skin retention of HC and TAC are presented in Tables 7 and 8, respectively. These data were arranged according to the vehicle class used. Within each class, data were ranked in the descending order of average percent of dose retained in the epidermis. The ranges within each group were calculated by dividing the highest retention by the lowest retention. Among the glycols, the epidermal and dermal retention ranges were almost similar for HC. A six fold range for epidermal and a nine fold range for dermal retention was observed for TAC. Interestingly propylene glycol ranked as the best vehicle for epidermal retention for both HC and TAC. The ranking of the epidermal and dermal retention was different for both HC and TAC.

Table 7. Ranking of Vehicles Classwise, According to the Percent of Dose of Hydrocortisone Retained in the Hairless Rat Skin Layers.

#	VEHICLES	PERCENT RETAINED ± S.D.		RANK	
		EPIDERMIS	DERMIS	A	B
1.	Propylene Glycol	4.75 ± 0.856	1.15 ± 0.533	1	2
2.	Ethylene Glycol	3.60 ± 1.97	1.09 ± 0.425	2	3
3.	Diethylene Glycol	2.80 ± 0.508	1.32 ± 0.317	3	1
4.	PEG 300	0.145 ± 0.119	0.0843 ± 0.0186	4	4
5.	PEG 200	0.106 ± 0.02	0.0613 ± 0.00808	5	5
6.	PEG 400	0.043 ± 0.0295	0.0570 ± 0.00346	6	6
7.	Isostearyl Alcohol	2.78 ± 0.320	3.82 ± 1.90	1	1
8.	Ethanol	1.36 ± 0.395	0.230 ± 0.0857	2	2
9.	Isopropanol	1.27 ± 0.302	0.142 ± 0.0305	3	3
10.	Isopropyl Palmitate	1.73 ± 0.534	2.24 ± 0.320	1	1
11.	Isopropyl Myristate	1.71 ± 0.847	1.58 ± 0.225	2	2
12.	Tributyrin	1.63 ± 0.391	0.779 ± 0.220	1	2
13.	Tricaprylin	1.03 ± 0.124	1.13 ± 0.154	2	1
14.	Triolein	0.646 ± 0.179	0.258 ± 0.0311	3	4
15.	Monoacetin	0.621 ± 0.116	0.319 ± 0.159	4	3
16.	Diacetin	0.145 ± 0.00165	0.198 ± 0.0991	5	5
17.	Triacetin	0.129 ± 0.0323	0.137 ± 0.0578	6	6
18.	Miglyol 812^{R}	3.52 ± 1.02	1.99 ± 0.652	1	3
19.	Miglyol 840^{R}	2.41 ± 0.226	3.06 ± 0.398	2	1
20.	Imwitor 408^{R}	3.56 ± 1.67	2.53 ± 0.459	3	2
21.	Imwitor 412^{R}	1.52 ± 0.511	1.17 ± 0.136	4	4
22.	Dimethyl Sulfoxide	3.60 ± 1.03	0.838 ± 0.483	1	4
23.	Oleic Acid	1.55 ± 0.249	1.84 ± 0.150	2	1
24.	Azone^{R}	0.970 ± 0.260	1.40 ± 0.0709	3	3
25.	Linoleic Acid	0.815 ± 0.617	1.60 ± 0.542	4	2
26.	Transcutol^{R}	0.567 ± 0.141	0.640 ± 0.100	5	5

Rank A: Ranking of Vehicles by % of Dose Retained in Epidermis
Rank B: Ranking by Vehicles by % of Dose Retained in Dermis

Table 8. Ranking of Vehicles Classwise, According to the Percent of Dose of Triamcinolone Acetonide Retained in the Hairless Rat Skin Layers.

#'s	VEHICLES	PERCENT RETAINED ± S.D.		RANK	
		EPIDERMIS	DERMIS	A	B
1	Propylene Glycol	5.09 ± 1.47	0.769 ± 0.422	1	2
2.	Ethylene Glycol	4.41 ± 1.37	1.61 ± 1.09	2	1
3.	Diethylene Glycol	0.857 ± 0.246	0.182 ± 0.0767	3	3
4.	PEG 200	0.110 ± 0.0437	0.0707 ± 0.0422	4	4
5.	PEG 300	0.0423 ± 0.0150	0.0463 ± 0.00850	5	6
6.	PEG 400	0.011 ± 0.000954	0.0473 ± 0.00451	6	5
7.	Isopropanol	0.885 ± 0.417	0.172 ± 0.0359	1	3
8.	Ethanol	0.755 ± 0.273	0.311 ± 0.0945	2	2
9.	Isostearyl Alcohol	0.452 ± 0.156	0.721 ± 0.206	3	1
10.	Isopropyl Palmitate	1.92 ± 0.249	3.71 ± 2.80	1	1
11.	Isopropyl Myristate	1.84 ± 0.526	1.89 ± 0.131	2	2
12.	Triolein	2.18 ± 1.32	0.809 ± 0.268	1	2
13.	Tributyrin	1.64 ± 0.442	1.09 ± 0.107	2	1
14.	Tricaprylin	1.62 ± 0.0961	0.699 ± 0.0862	3	3
15.	Monoacetin	0.640 ± 0.532	0.203 ± 0.0777	4	4
16.	Triacetin	0.157 ± 0.0661	0.196 ± 0.0480	5	5
17.	Diacetin	0.0643 ± 0.0240	0.0440 ± 0.0137	6	6
18.	Miglyol 840^{R}	2.44 ± 0.373	2.98 ± 0.255	1	1
19.	Miglyol 812^{R}	1.07 ± 0.302	1.56 ± 0.589	3	4
20.	Imwitor 408^{R}	1.85 ± 0.762	2.50 ± 0.709	2	2
21.	Imwitor 412^{R}	0.975 ± 0.370	1.84 ± 0.272	4	3
22.	Dimethyl Sulfoxide	2.21 ± 0.622	0.525 ± 0.103	1	5
23.	Transcutol^{R}	1.06 ± 0.267	0.614 ± 0.190	2	4
24.	Azone^{R}	0.967 ± 0.216	1.12 ± 0.144	3	3
25.	Linoleic Acid	0.898 ± 0.0835	1.66 ± 0.441	4	2
26.	Oleic Acid	0.682 ± 0.214	1.77 ± 0.514	5	1

Rank A: Ranking of Vehicles by % of Dose Retained in Epidermis
Rank B: Ranking by Vehicles by % of Dose Retained in Dermis

For the polyglycol vehicles, a range of five fold for epidermal and a 1.5 fold dermal retention was observed in the case of HC while a 10 fold range for the epidermal and a 1.5 fold range for the dermal retention was observed for TAC. PEG 400 provided the lowest epidermal and dermal retention of HC and TAC. Similar findings have been reported for other drugs such as a PAF Antagonist and a lipoxygenase inhibitor[19,22,23]. Reasons for such a lack of drug delivery capability of PEG 400 are not clear. In spite of such property of PEG 400 it is still widely used in dermatological formulations.

The results obtained with the three alcohols tested are of interest. Ethanol and isopropanol showed comparable epidermal retention of HC. The epidermal retention values of HC obtained with isostearyl alcohol were about twice as high as with ethanol or isopropanol. The dermal retention values for HC obtained with isostearyl alcohol were over 15-20 fold higher than those obtained with ethanol and isopropanol. The dermal retention of HC from isostearyl alcohol was substantially higher than that from either ethanol (16.6 fold higher) or from isopropanol (26.9 fold higher). These interesting data suggest that isostearyl alcohol is a much superior vehicle for HC than TAC because of the substantially higher epidermal and dermal retention values obtained, 6 fold higher epidermal and 5 fold higher dermal retention values. TAC showed comparable epidermal and dermal retention, however, the epidermal

retention values for TAC with isostearyl alcohol were about half of those obtained with ethanol and isopropanol. The dermal retention of TAC doubled in going from isopropanol to ethanol and doubled further from isostearyl alcohol.

The two esters evaluated, isopropyl palmitate and isopropyl myristate provided comparable epidermal retention for both HC and TAC. The dermal retention observed with palmitate was about 1.4 fold higher than with myristate for HC and about 2 fold higher for TAC. In general, both esters appear to be good vehicles for the two drugs investigated.

The epidermal and dermal retention values of HC and TAC obtained with the six glyceride vehicles studied are generally lower than those seen with the vehicles discussed thus far. Ranges of 13 fold epidermal and 8 fold dermal retention were noted for HC. These ranges for TAC were substantially different, 34 fold for the epidermal and 25 fold for the dermal retention. Generally, for a given vehicle tested, higher retention was observed for TAC than for HC. Overall, it appears that the glyceride vehicles may not be optimal vehicles for dermatological formulations. However, it should be said with caution because, as mentioned earlier, vehicle effects can be and often are compound dependent and only two drugs have been evaluated in this study.

Four mixed commercial vehicles tested included two miglyols and two imwitors. Miglyols[R] contain propylene glycol caprylate/dicaprylate. Imwitors[R] contain propylene glycol dilaurates. The overall ranges of epidermal and dermal retention values for HC and TAC are about 2 to 2.6, indicating comparable effects of these vehicles on the local drug delivery. However, data show mixed results in terms of vehicle effects on HC versus TAC. For example, epidermal and dermal retentions of HC and TAC from Miglyol 840[R] were comparable. But, Miglyol 812[R] provided over 3 fold higher epidermal and about 1.3 fold higher dermal retention of HC. Imwitor 412[R] showed a flip-flop behavior. It provided 1.5 fold higher epidermal but, 1.6 fold lower dermal retention of HC than TAC. Imwitor 408[R], on the other hand, showed almost twice epidermal retention but, comparable dermal retention. These vehicles appear to be efficient in providing local drug delivery. The magnitudes of retentions obtained are on the higher side when considering all 26 vehicles evaluated. There is an interesting feature which these mixed commercial vehicles offer. They seem to provide high epidermal as well as high dermal retention along with less than 5 fold difference between the retention in the two layers for both drugs studied. While it may be a desirable feature, note that it does not happen with all drugs. For example, the ratios of epidermal to dermal retentions of Ro 24-5413 and Ro 24-0238 were reported to be far in excess of five [22,23].

Five commonly known enhancers were also evaluated as vehicles in the present study. The ranges of epidermal and dermal retentions from these vehicles were found to be 6.3 fold and 2.8 fold for HC, respectively and 3.2 fold and 3.4 fold for TAC, respectively. Apparently, these enhancers do not seem to affect the local drug delivery of HC and TAC similarly. DMSO showed a rather large difference with regard to the epidermal versus dermal retention values, about 4.2 fold higher retention in the epidermis than dermis for both drugs tested. This ratio is in the range of 0.39 fold to 1.7 fold for the other four enhancers, oleic acid, Azone^{R}, linoleic acid and Transcutol^{R}. The results obtained with DMSO are rather disappointing with regard to the dermal retention observed. It is probably the most widely studied and "admired" enhancer as reported in the literature. But, a careful analysis indicates that such "sentiments" about DMSO were primarily derived from permeation/flux data as opposed to retention data. Actually, there are virtually no reports on the utility of DMSO in enhancing local drug delivery.

Oleic acid has been fairly well studied enhancer. However, almost all of the literature reports are about the enhancing effects on drug permeation/flux as opposed to drug retention. It provided an equal retention of HC in the epidermal and dermal layers. But, for TAC, it showed 2.5 fold higher retention in the dermal layer.

Azone^{R} is an another extensively studied compound for its effects on drug permeation. It has been found to enhance the permeation of a vast number of compounds[31,195]. Its effects on the epidermal and dermal retention of HC and TAC, however, were found to be only modest. The effects of Transcutol^{R} on the epidermal and dermal retentions of HC and TAC were observed to be somewhat lower than Azone^{R}. The effects of linoleic acid on the local delivery of HC and TAC were also found to be modest. The dermal delivery was noted to be about twice as high as the epidermal delivery.

Retention data for HC and TAC from all vehicles are ranked in Tables 9 and 10, respectively. The vehicles were ranked in a descending order of the epidermal retention. The corresponding dermal rank orders are also provided in the same tables. The epidermal retention values vary over ranges of 110 fold and 463 fold for HC and TAC, respectively. The corresponding dermal retention ranges are 67 fold and 84 fold for HC and TAC, respectively. These are extremely large ranges observed and point to the strength of vehicle effects on local drug delivery. For both drugs, propylene glycol provided maximal and PEG 400 provided minimal epidermal retention. This observation is of special importance because both of these vehicles are quite commonly used in dermatological formulations.

The top five vehicles for HC retention on the basis of the mean retention values in the epidermis are propylene glycol, DMSO, ethylene glycol, Imwitor 408 and Miglyol 812^{R}, and for the dermal retention, isostearyl alcohol, Miglyol 840^{R}, Imwitor 408^{R}, isopropyl palmitate and Miglyol 812^{R} (Tables 9 and 10). The top five vehicles for retention of TAC on the basis of the mean retention values in the epidermis are propylene glycol, ethylene glycol, DMSO, triolein and Miglyol 840, and for the dermal retention, they are isopropyl palmitate, Miglyol 840^{R}, Imwitor 408^{R}, isopropyl myristate and Imwitor 412^{R}.

Table 9. Ranking of All Vehicles Tested According to the Percentage of Dose of Hydrocortisone Retained in the Hairless Rat Epidermal Skin Layer.

#	VEHICLES	PERCENT RETAINED ± S.D.		RANK	
		EPIDERMIS	DERMIS	A	B
1.	Propylene Glycol	4.75 ± 0.856	1.15 ± 0.533	1	12
2.	Dimethyl Sulfoxide	3.60 ± 1.03	0.838 ± 0.483	2	15
3.	Ethylene Glycol	3.60 ± 1.97	1.09 ± 0.425	3	14
4.	Imwitor 408®	3.56 ± 1.67	2.53 ± 0.459	4	3
5.	Miglyol 812®	3.52 ± 1.02	1.99 ± 0.652	5	5
6.	Diethylene Glycol	2.80 ± 0.508	1.32 ± 0.317	6	10
7.	Isostearyl Alcohol	2.78 ± 0.320	3.82 ± 1.90	7	1
8.	Miglyol 840®	2.41 ± 0.226	3.06 ± 0.398	8	2
9.	Isopropyl Palmitate	1.73 ± 0.534	2.24 ± 0.320	9	4
10.	Isopropyl Myristate	1.71 ± 0.847	1.58 ± 0.225	10	8
11.	Tributyrin	1.63 ± 0.391	0.779 ± 0.220	11	16
12.	Oleic Acid	1.55 ± 0.249	1.84 ± 0.150	12	6
13.	Imwitor 412®	1.52 ± 0.511	1.17 ± 0.136	13	11
14.	Ethanol	1.36 ± 0.395	0.230 ± 0.0857	14	20
15.	Isopropanol	1.27 ± 0.302	0.142 ± 0.0305	15	22
16.	Tricaprylin	1.03 ± 0.124	1.13 ± 0.154	16	13
17.	Azone®	0.970 ± 0.260	1.40 ± 0.0709	17	9
18.	Linoleic Acid	0.815 ± 0.617	1.60 ± 0.542	18	7
19.	Triolein	0.646 ± 0.179	0.258 ± 0.0311	19	19
20.	Monoacetin	0.621 ± 0.116	0.319 ± 0.159	20	18
21.	Transcutol®	0.567 ± 0.141	0.640 ± 0.100	21	17
22.	Diacetin	0.145 ± 0.00165	0.198 ± 0.0991	22	21
23.	PEG 300	0.145 ± 0.119	0.0843 ± 0.0186	23	24
24.	Triacetin	0.129 ± 0.0323	0.137 ± 0.0578	24	23
25.	PEG 200	0.106 ± 0.02	0.0613 ± 0.00808	25	25
26.	PEG 400	0.043 ± 0.0295	0.0570 ± 0.00346	26	26

Rank A: Ranking of Vehicles by % of Dose Retained in Epidermis
Rank B: Ranking by Vehicles by % of Dose Retained in Dermis

Table 10. Ranking of All Vehicles Tested According to the Percentage of Dose of Triamcinolone Acetonide Retained in the Hairless Rat Epidermal Skin Layer.

#'s	VEHICLES	PERCENTAGE OF DOSE RETAINED ± S.D.		RANK	
		EPIDERMIS	DERMIS	A	B
1.	Propylene Glycol	5.09 ± 1.47	0.769 ± 0.422	1	13
2.	Ethylene Glycol	4.41 ± 1.37	1.61 ± 1.09	2	8
3.	Dimethyl Sulfoxide	2.21 ± 0.622	0.525 ± 0.103	3	17
5.	Miglyol 840^{R}	2.44 ± 0.373	2.98 ± 0.255	5	2
4.	Triolein	2.18 ± 1.32	0.809 ± 0.268	4	12
6.	Isopropyl Palmitate	1.92 ± 0.249	3.71 ± 2.80	6	1
7.	Imwitor 408^{R}	1.85 ± 0.762	2.50 ± 0.709	7	3
8.	Isopropyl Myristate	1.84 ± 0.526	1.89 ± 0.131	8	4
9.	Tributyrin	1.64 ± 0.442	1.09 ± 0.107	9	11
10.	Tricaprylin	1.62 ± 0.0961	0.699 ± 0.0862	10	15
11.	Miglyol 812^{R}	1.07 ± 0.302	1.56 ± 0.589	11	9
12.	Transcutol^{R}	1.06 ± 0.267	0.614 ± 0.190	12	16
13.	Imwitor 412^{R}	0.975 ± 0.370	1.84 ± 0.272	13	5
14.	Azone^{R}	0.967 ± 0.216	1.12 ± 0.144	14	10
15.	Linoleic Acid	0.898 ± 0.0835	1.66 ± 0.441	15	7
16.	Isopropanol	0.885 ± 0.417	0.172 ± 0.0359	16	22
17.	Diethylene Glycol	0.857 ± 0.246	0.182 ± 0.0767	17	21
18.	Ethanol	0.755 ± 0.273	0.311 ± 0.0945	18	18
19.	Oleic Acid	0.682 ± 0.214	1.77 ± 0.514	19	6
20.	Monoacetin	0.640 ± 0.532	0.203 ± 0.0777	20	19
21.	Isostearyl Alcohol	0.452 ± 0.156	0.721 ± 0.206	21	14
22.	Triacetin	0.157 ± 0.0661	0.196 ± 0.0480	22	20
23.	PEG 200	0.110 ± 0.0437	0.0707 ± 0.0422	23	23
24.	Diacetin	0.0643 ± 0.0240	0.0440 ± 0.0137	24	26
25.	PEG 300	0.0423 ± 0.0150	0.0463 ± 0.00850	25	25
26.	PEG 400	0.011 ± 0.000954	0.0473 ± 0.00451	26	24

Rank A: Ranking of Vehicles by % of Dose Retained in Epidermis
Rank B: Ranking by Vehicles by % of Dose Retained in Dermis

These observations indicate the following:

a. Within a given skin layer, epidermis or dermis, drug retention was dependent on the drug used.

b. For a given drug, the ranking of vehicles was not same for epidermis and dermis.

c. The ranges of the epidermal and dermal retention for either drug are substantially large and underscore the critical importance of proper vehicle selection.

d. Reasons for such diverse effects are not clear at this time. However, the clue may lie in the fact that the vehicles themselves are absorbed in the skin layers. This would change the "microenvironment" in the tissue and the thermodynamic rules of transport processes will not be followed. How changes in the microenvironment affect drug delivery is a complex issue and elucidation of possible mechanisms is out of the scope of this thesis.

4.3.2. Drug Retention and Lipophilicity: The two drugs HC and TAC, belong to the same therapeutic class and both are glucocorticoids used as anti-inflammatory agents having comparable molecular weights. These compounds, however, differ with respect to solubility in water and partition coefficients. HC is more water soluble than TAC and has a lower ether/water partition coefficient, e.g. 1.3 vs 13.1. These properties may indicate that the skin uptake may be distinguishable. As it is apparent from the foregoing discussion, this is not the case. Also, the solubility of HC and TAC in the vehicles studied is not distinguishable. The reasons for the skin uptake data not to follow the drugs' partition coefficients, are not clear.

4.3.3. Drug Retention and Solubility: The ranges of solubility values of HC and TAC was found to be, 1912 and 2297, respectively, in the 26 vehicles studied. The ranges observed in the epidermal and dermal retentions of HC are 110 and 67, respectively (Table 9). For TAC, the ranges of epidermal and dermal retentions are 462 and 84, respectively (Table 10). To further examine the relationship between retention and solubility, the epidermal and dermal retention data were plotted as a function of solubility separately for each class of vehicles studied for both drugs (Figures 6-12). Each of these figures contains four plots, A-D, which include epidermal and dermal plots for both drugs studied.

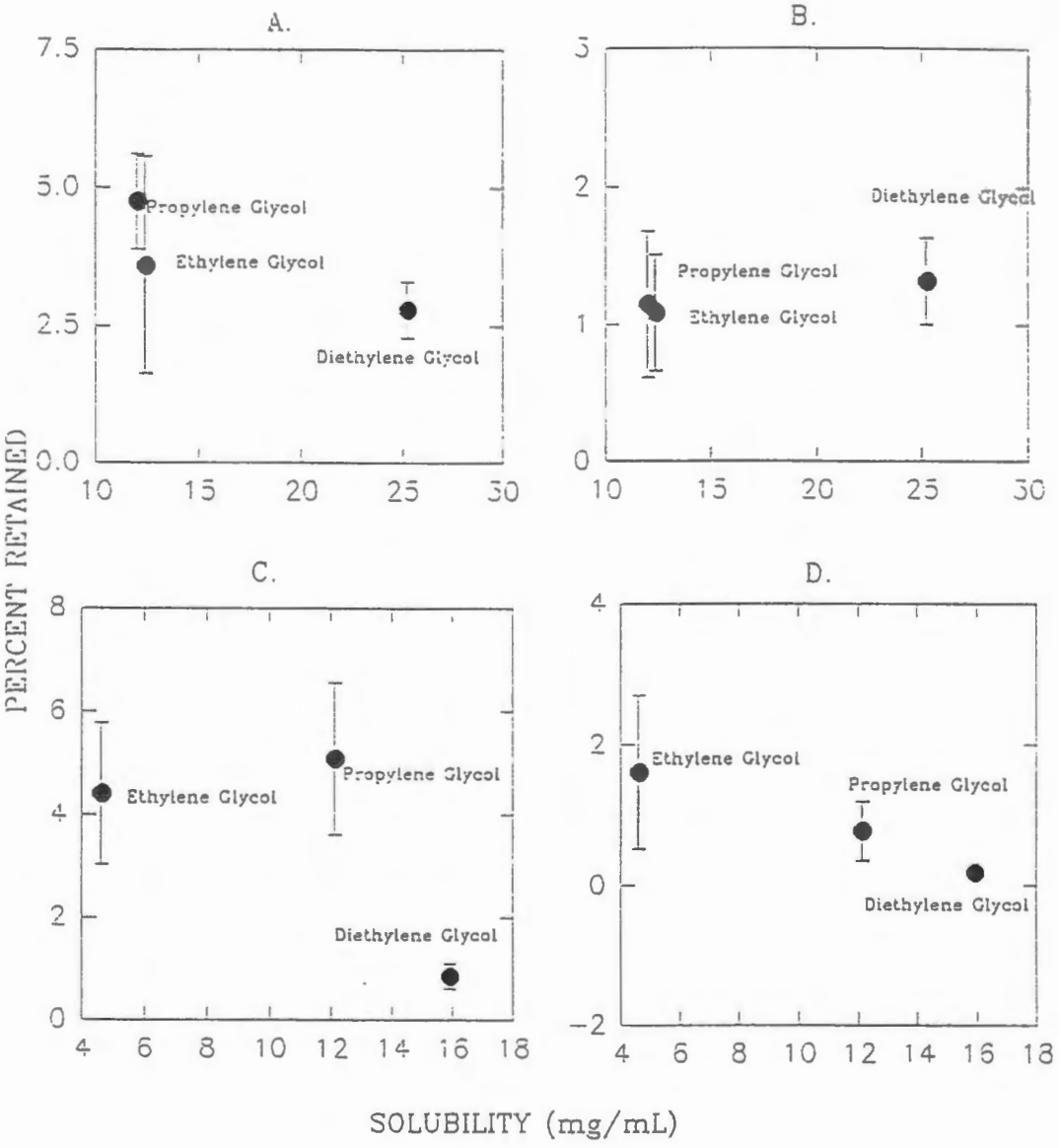


Figure 6. Percent Retention vs Solubility Plots of Hydrocortisone(HC) and Triamcinolone Acetonide(TAC) in the Epidermis and Dermis for the Three "Glycol" Vehicles Studied in the In Vivo Hairless Rat Model.

A: HC in Epidermis B: HC in Dermis

C: TAC in Epidermis D: TAC in Dermis

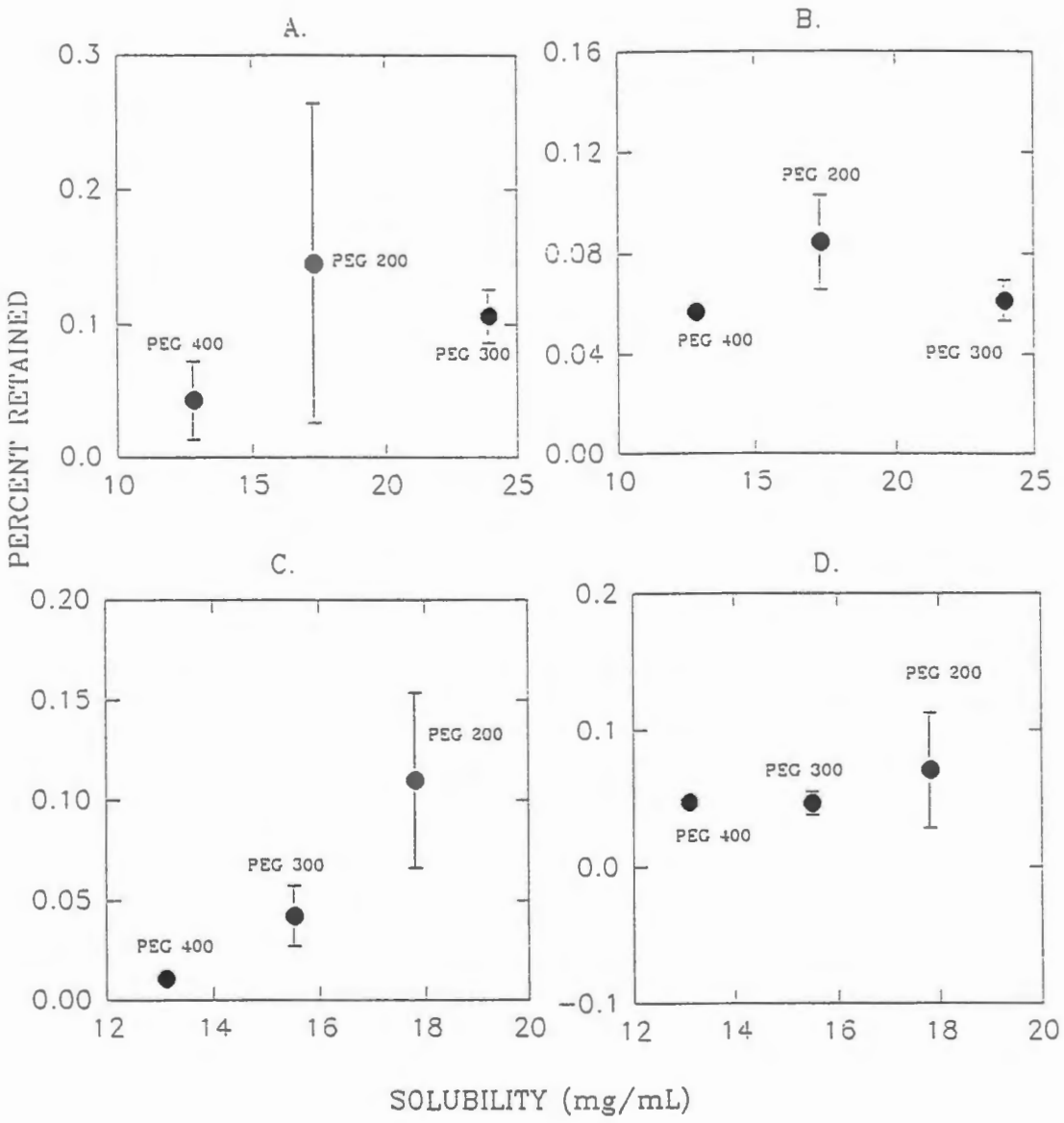


Figure 7. Percent Retention vs Solubility Plots of Hydrocortisone(HC) and Triamcinolone Acetonide(TAC) in the Epidermis and Dermis for the Three "Polyglycol" Vehicles Studied in the In Vivo Hairless Rat Model.

A: HC in Epidermis B: HC in Dermis

C: TAC in Epidermis D: TAC in Dermis

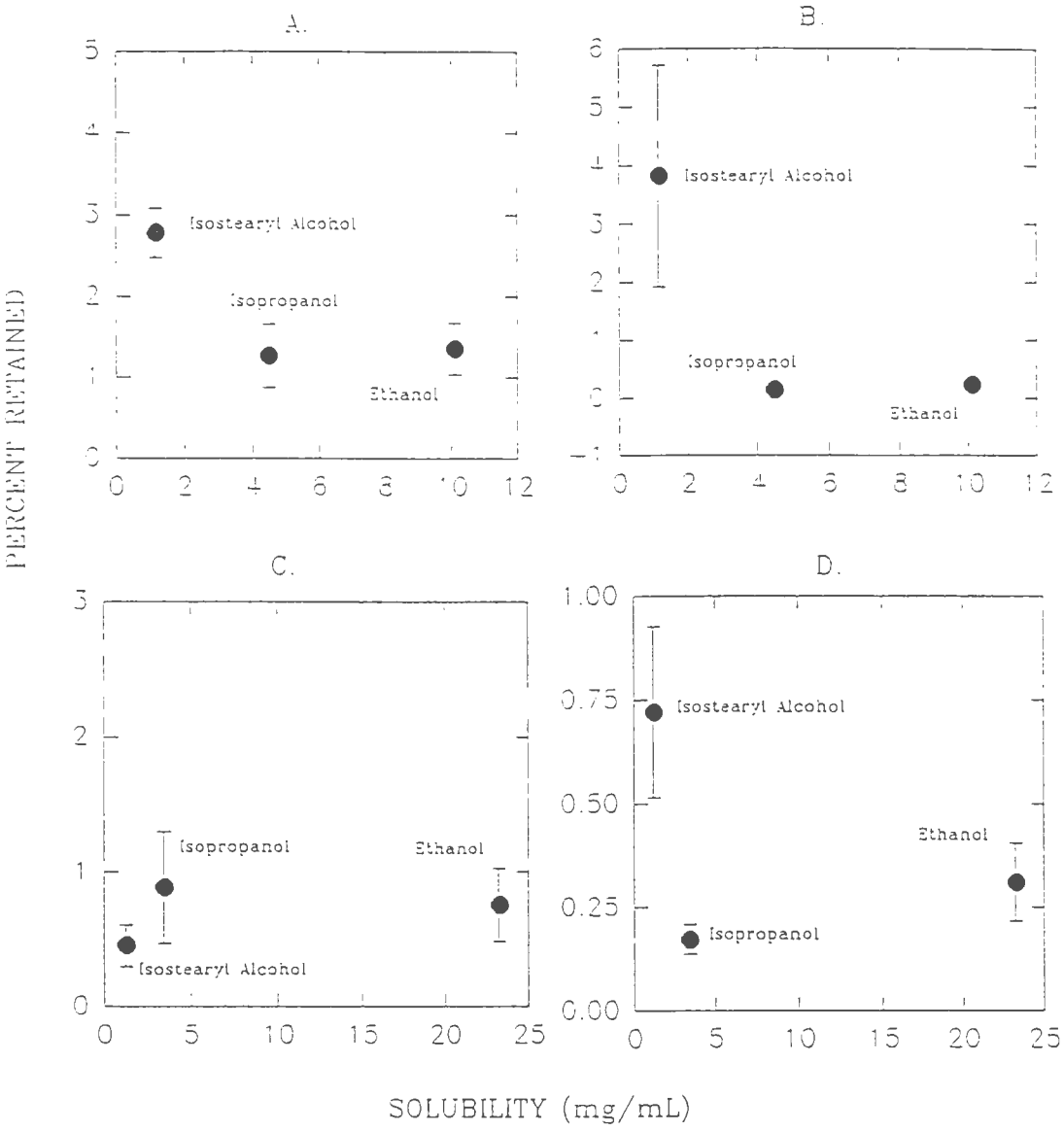


Figure 8. Percent Retention vs Solubility Plots of Hydrocortisone(HC) and Triamcinolone Acetonide(TAC) in the Epidermis and Dermis for the Three "Alcohol" Vehicles Studied in the In Vivo Hairless Rat Model.

A: HC in Epidermis B: HC in Dermis

C: TAC in Epidermis D: TAC in Dermis

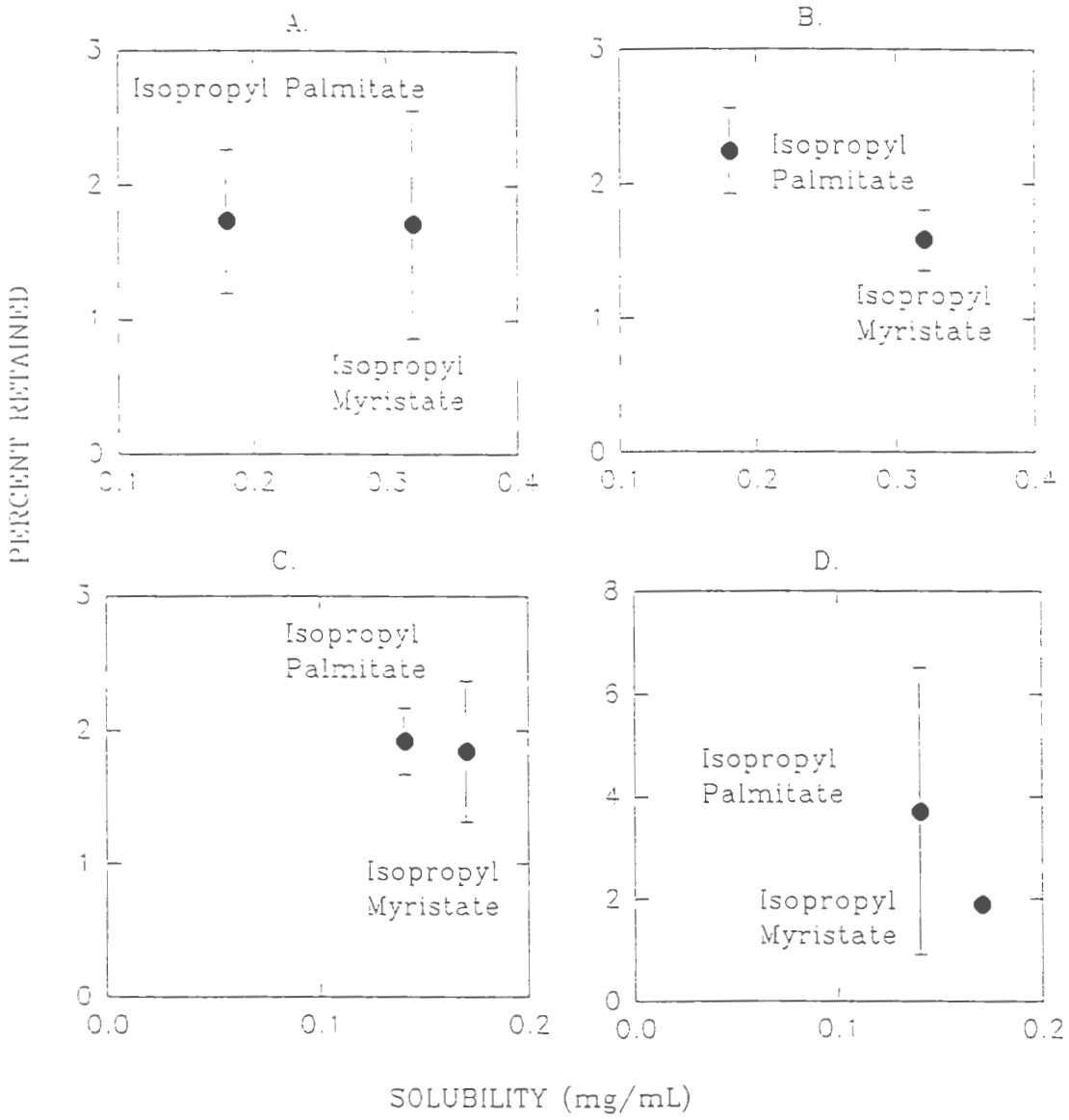


Figure 9. Percent Retention vs Solubility Plots of Hydrocortisone(HC) and Triamcinolone Acetonide(TAC) in the Epidermis and Dermis for the Three "Ester" Vehicles Studied in the In Vivo Hairless Rat Model.

A: HC in Epidermis B: HC in Dermis

C: TAC in Epidermis D: TAC in Dermis

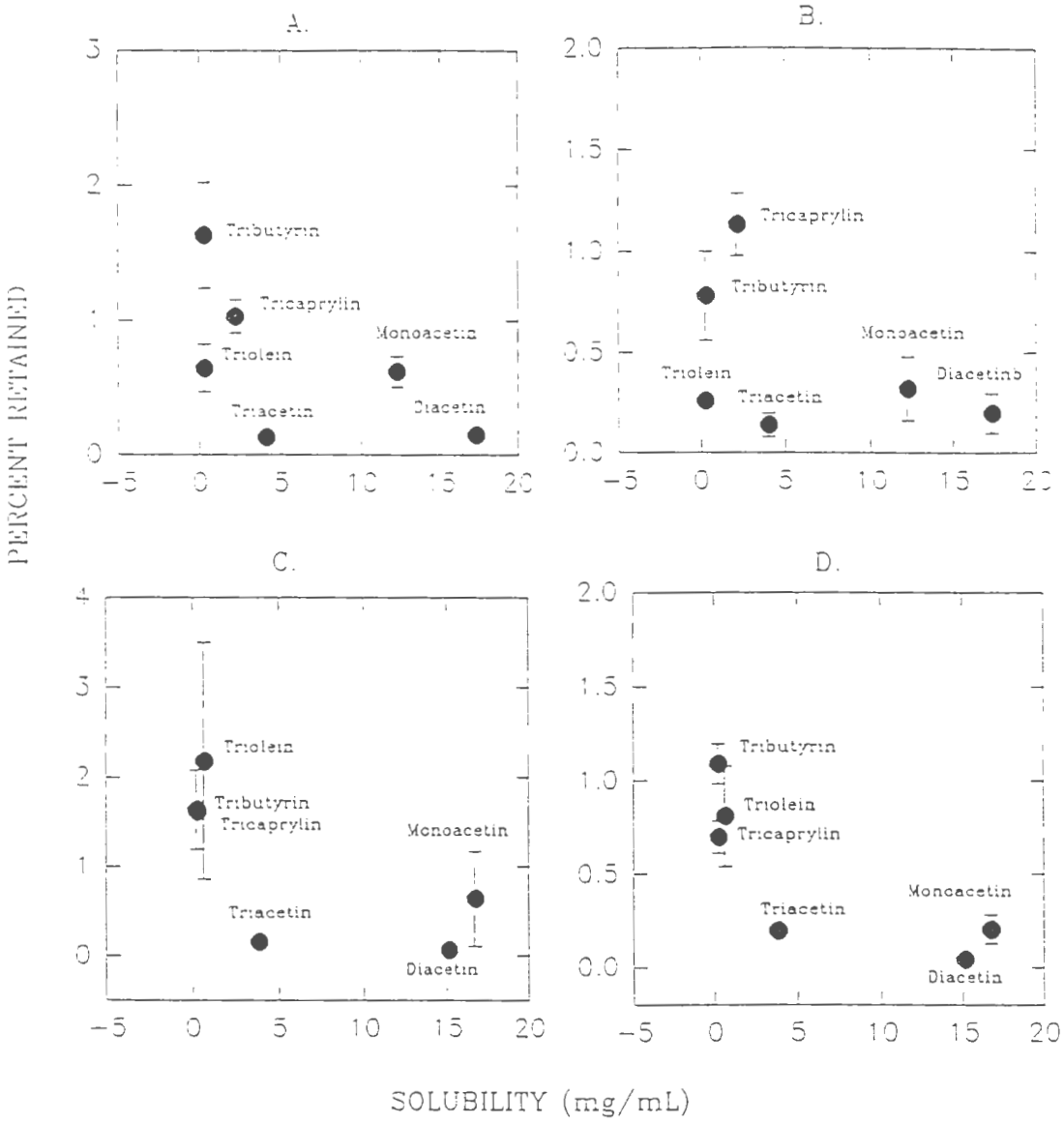


Figure 10. Percent Retention vs Solubility Plots of Hydrocortisone(HC) and Triamcinolone Acetonide(TAC) in the Epidermis and Dermis for the Six "Glycerides" Vehicles Studied in the In Vivo Hairless Rat Model.

A: HC in Epidermis B: HC in Dermis

C: TAC in Epidermis D; TAC in Dermis

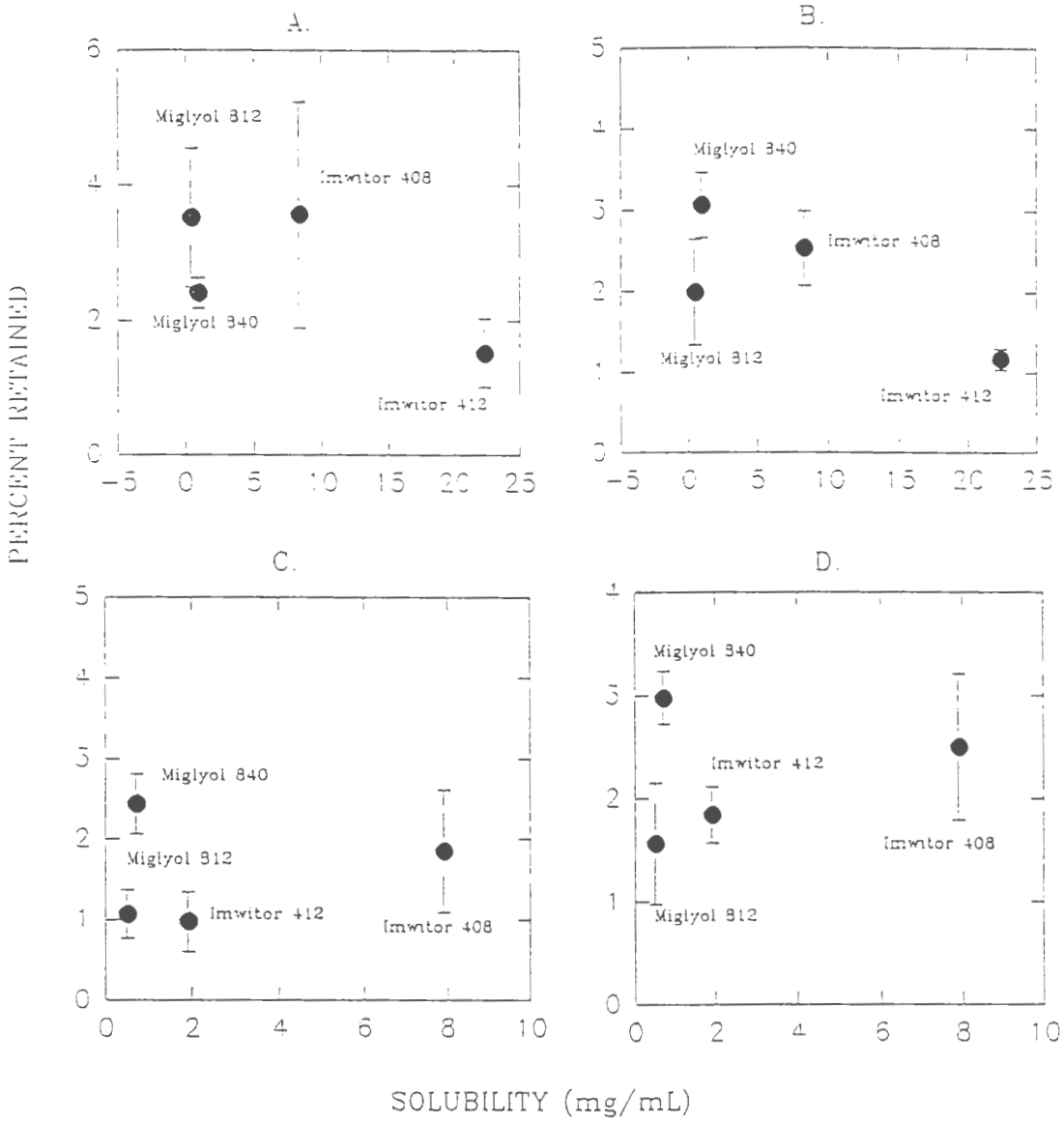


Figure 11. Percent Retention vs Solubility Plots of Hydrocortisone(HC) and Triamcinolone Acetonide(TAC) in the Epidermis and Dermis for the Four "Commercial" Vehicles Studied in the In Vivo Hairless Rat Model.

A: HC in Epidermis B: HC in Dermis

C: TAC in Epidermis D: TAC in Dermis

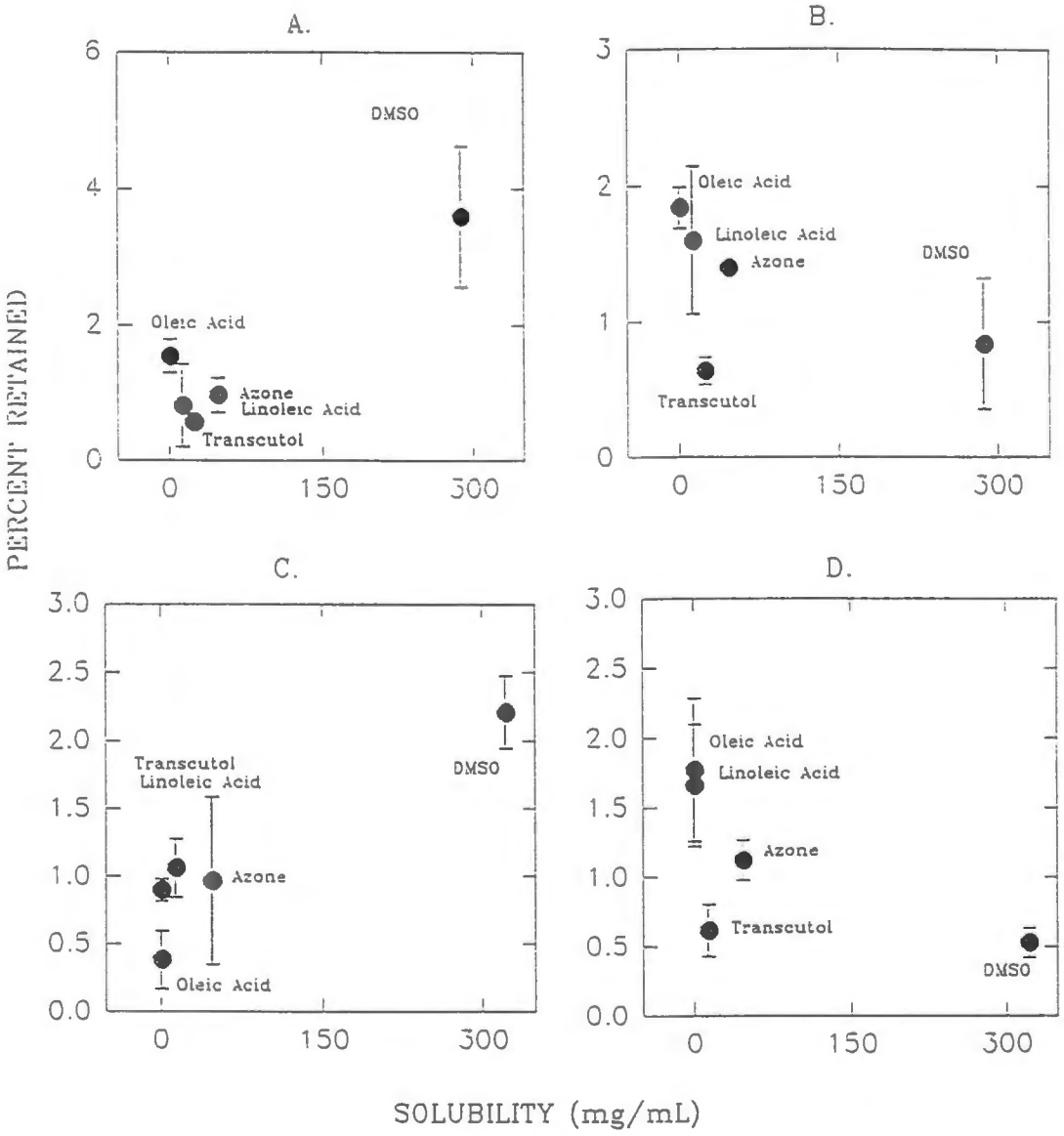


Figure 12. Percent Retention vs Solubility Plots of Hydrocortisone(HC) and Triamcinolone Acetonide(TAC) in the Epidermis and Dermis for the Five "Enhancers" Vehicles Studied in the In Vivo Hairless Rat Model.

A: HC in Epidermis B: HC in Dermis

C: TAC in Epidermis D: TAC in Dermis

Figure 6 contains plots for the three glycol vehicles tested. The epidermal retention of HC showed a decline with solubility whereas the dermal retention showed an increase. The trend for the epidermal retention of TAC was an initial increase in retention followed by a decrease. This trend for the dermal retention was almost a linear decline with solubility. In addition to these differing trends, the rank order of the retention in epidermis vs dermis was also different for the two drugs.

The retention values of three polyglycol vehicles were plotted against the solubilities in Figure 7. The trend in the epidermal retention of HC was similar to that in the dermis, both layers show an initial increase followed by a decrease in the drug uptake. The rank order for the three vehicles was also same. The situation is different for TAC where the epidermal retention showed a consistent increase in going from PEG 400 through PEG 300 to PEG 200. The dermal retention for PEG 400 was comparable to that from PEG 300. Retention from PEG 200 was about 50 % higher than the other PEGs.

Similarly, the retention versus solubility data for the three alcohols was plotted in Figure 8. The epidermal retention of HC declined sharply in going from isostearyl alcohol to isopropanol and remained invariant from ethanol. A similar trend was observed for the dermal retention except that the

decline from isostearyl alcohol to isopropanol was much sharper. The trend observed for the epidermal retention of TAC was different. The retention first increased in going from isostearyl alcohol to isopropanol followed by a decrease from ethanol. The dermal trend for TAC was opposite to the epidermal trend.

Figure 9 contains plots for the two esters studied. The epidermal retention of HC remained invariant in going from isopropyl palmitate to isopropyl myristate but, a decrease in the dermal retention was noted. For TAC, the epidermal retention was comparable for the two vehicles but, the dermal retention showed almost a doubling of the dermal retention in going from isopropyl myristate to isopropyl palmitate.

Plots of skin layer retention versus solubility were made for the six glyceride vehicles studied (Figure 10). The epidermal and dermal retention showed a general decrease with solubility for both drugs studied. However, the rank orders of the vehicles was different between the epidermal and dermal retention for both drugs.

Figure 11 contains plots for the four mixed commercial vehicles studied. At first glance, the epidermal and dermal retention of HC seem to show a decrease with solubility. Upon further examination, it became clear that the epidermal

retention first decreased in going from Miglyol 812^{R} to Miglyol 840^{R} followed by an increase from Imwitor 408^{R} and a decrease from Imwitor 412^{R}. The dermal retention behaved differently in that initially there was an increase in going from Miglyol 812^{R} to Miglyol 840^{R} followed by an almost linear decrease in going from Miglyol 840^{R} through Imwitor 408^{R} to Imwitor 412^{R}. For TAC, the epidermal and dermal retention showed an initial decrease in going from Miglyol 840^{R} to Miglyol 812^{R} followed by an increase, however, the rank orders of the vehicles were not same.

Finally the plot of retention versus solubility were made for the five enhancer vehicles studied (Figure 12). For both, HC and TAC, general increase in the epidermal retention was observed with solubility, DMSO showing the maximal retention. The rank orders of the vehicles were not same. The dermal retention showed a decrease with solubility, transcutol^{R} and DMSO showing lowest values.

The trends discussed above between drug retention in epidermis and dermis, and drug solubility are quite interesting and revealing. As can be seen, no definite trends were observed. For most part, epidermis behaved differently from the dermis and HC behaved differently from TAC with regard to epidermis and/or dermis. Therefore, it would appear that drug solubility alone can not aid in predicting drug uptake in the skin

layers. This emphasizes the point raised earlier[22,23] as to the importance of the uptake of vehicles in the epidermis and dermis. Vehicles certainly permeate into the skin and are retained there. This changes the "microenvironment" in the skin which is certain to alter drug retention. Until more thorough studies are carried out to better understand the complex drug/vehicle/skin interactions, it probably would not be feasible to make predictions on what vehicles would provide optimal drug delivery.

4.3.4. Criteria for Formulation Selection- Retention vs Flux: Some information was presented in the Introduction Section of this thesis on the relevance of drug uptake in the skin layers as a criterion of choice over flux. Data gathered in this study indirectly support the contention that flux data do not necessarily provide correct information for selecting vehicles for dermatological formulations. To emphasize this point, ratios of epidermal to dermal retention were computed and ranked in a descending order. These results are presented in Tables 11 and 12 for HC and TAC, respectively.

The ratios range from 8.94 to 0.510 , a range of about 18 fold, for HC and 6.62 to 0.223, a range of about 30 fold, for TAC. Vehicles showing ratios of 1 or less provide better dermal delivery of drugs. It is an important observation because for certain diseases, a better dermal delivery may be needed. The rank orders of the ratios for the two drugs are

not same. This strongly indicates that different vehicles affect the skin layers differently and provide varying tissue drug concentrations. To use flux values for screening formulations, the ratios obtained should have been constant. Results obtained in this study show that there is need to experimentally determine drug retention in the skin layers to screen dermatological formulations for topical drug delivery.

4.3.5 Utility of the In Vivo Model for Screening Vehicles-Significance of the Present Study: Dermatological formulations often contain several excipients. As seen in Table 3, a formulator needs to select his excipients from a rather large list. Until recently, when local drug delivery was recognized as a criterion for formulation selection, the only available criteria included cosmetic acceptability, lack of irritation of the product and stability of the drug. Given the current state of knowledge and awareness, one can make more judicious choices of vehicles/excipients based on data on drug delivery. The in vivo hairless rat model used in this study can be useful in this regard. Many pertinent vehicles can be screened readily for their effects on drug retention in the desired skin layer and vehicles which provide maximal delivery can be selected for formulation development. Unfortunately, no predictive "tools" are yet available to predict optimal vehicles. One has to experimentally screen all potential vehicles. This becomes quite clear from data presented in this thesis. A given vehicle may be effective for a certain drug and not for another. It may be a good vehicle for providing optimal epidermal retention and not dermal retention. Until the state of the art advances in this area, a reliance on the experimental observations will have to continue.

5.0 SUMMARY AND CONCLUSIONS

An *in vivo* hairless rat model was used in this study to evaluate the effects of vehicles on the local drug delivery to the skin layers. For this purpose, two model drugs, ^{3}H-hydrocortisone (HC) and ^{3}H-triamcinolone acetonide (TAC), and 26 vehicles were investigated. These drugs were chosen due to their differing lipophilicities. The vehicles selected belonged to seven different classes, e.g. glycols, polyglycols, alcohols, esters, glycerides, mixed commercial vehicles and enhancer vehicles.

The solubility of HC and TAC was determined in all 26 vehicles chosen. A high performance liquid chromatography (HPLC) method was established for analyzing drug concentrations in the solubility samples. The method adopted has the advantage that it provided interference free results for 26 different vehicles, for two different drugs. The solubility data showed rather large ranges, 1912 fold for HC and 2297 fold for TAC indicating the value of selecting these vehicles. Such large solubility differences, when compared with the observed respective drug uptake data, was expected to reveal a lot of useful information.

Formulations of HC and TAC were prepared in vehicles at half of the respective solubilities. The formulations contained

both radiolabelled (^{3}H-labelled) and regular forms of the drugs. The formulations were applied on the animal's skin in a "finite dose" manner which amounted to 2 μL of the drug solution applied per cm^2 of the skin.

The animal model used was an in vivo hairless rat model similar to what has been used at Hoffmann-La Roche, Inc. for screening dermatological formulations for drug delivery. The formulation was applied on the abdominal skin of anesthetized rats for a three hour period. At that time, the excess formulation was wiped off the skin by using a "top wash" procedure. The animal was sacrificed and the skin was excised and sectioned into the epidermis and dermis and weighed. The drug was extracted from the skin layers by using a tissue oxidizer and analyzed by using a liquid scintillation counter. The drug uptake in the tissue was expressed in terms of drug concentration per gram of the tissue and in terms of percent of the applied dose retained in the skin layers.

The drug retention data were ranked in a descending order of percent epidermal and dermal retention values. The percent epidermal to dermal retention ratios were computed. Also, the percent retention data were plotted as a function of drug solubility separately for the seven classes of vehicles used. Interpretations of these results indicate the following findings:

Table 11. Ratios of Epidermal to Dermal Retention of Hydrocortisone From Different Vehicles in Hairless Rat Skin.

#	Vehicles	Ratios of Epidermal to Dermal Retention	Ranking
1.	Isopropanol	9.04 ± 1.36	1
2.	Ethanol	6.59 ± 3.14	2
3.	Dimethyl Sulfoxide	6.12 ± 4.78	3
4.	Propylene Glycol	5.27 ± 3.69	4
5.	Ethylene Glycol	3.30 ± 1.08	5
6.	Triolein	2.55 ± 0.835	6
7.	Diethylene Glycol	2.24 ± 0.846	7
8.	Monoacetin	2.23 ± 0.897	8
9.	Tributyrin	2.13 ± 0.383	9
10.	Miglyol 812^{R}	1.89 ± 0.860	10
11.	PEG 300	1.75 ± 1.61	11
12.	PEG 200	1.73 ± 0.310	12
13.	Imwitor 408^{R}	1.50 ± 0.847	13
14.	Imwitor 412^{R}	1.32 ± 0.542	14
15.	Isopropyl Myristate	1.12 ± 0.626	15
16.	Triacetin	1.01 ± 0.292	16
17.	Tricaprylin	0.937 ± 0.247	17
18.	Transcutol^{R}	0.880 ± 0.150	18
19.	Diacetin	0.868 ± 0.421	19
20.	Isostearyl Alcohol	0.866 ± 0.422	20
21.	Oleic Acid	0.848 ± 0.186	21
22.	Miglyol 840^{R}	0.803 ± 0.171	22
23.	Isopropyl Palmitate	0.760 ± 0.166	23
24.	PEG 400	0.754 ± 0.488	24
25.	Azone^{R}	0.700 ± 0.209	25
26.	Linoleic Acid	0.468 ± 0.261	26

Table 12. Ratios of Epidermal to Dermal Retention of Triamcinolone Acetonide From Different Vehicles in Hairless Rat Skin.

#	VEHICLES	Ratios of Epidermal to Dermal Retention	Ranking
1.	Propylene Glycol	9.35 ± 7.52	1
2.	Isopropanol	5.37 ± 3.19	2
3.	Diethylene Glycol	5.01 ± 1.37	3
4.	Dimethyl Sulfoxide	4.25 ± 1.07	4
5.	Ethylene Glycol	4.16 ± 3.19	5
6.	Monoacetin	3.70 ± 3.95	6
7.	Triolein	2.56 ± 0.840	7
8.	Ethanol	2.41 ± 0.243	8
9.	Tricaprylin	2.34 ± 0.387	9
10.	PEG 200	2.00 ± 1.57	10
11.	Transcutol^{R}	1.98 ± 1.23	11
12.	Tributyrin	1.51 ± 0.405	12
13.	PEG 300	1.48 ± 1.17	13
14.	Diacetin	1.44 ± 0.151	14
15.	Isopropyl Myristate	0.968 ± 0.240	15
16.	Azone^{R}	0.876 ± 0.251	16
17.	Miglyol 840^{R}	0.821 ± 0.113	17
18.	Imwitor 408^{R}	0.805 ± 0.490	18
19.	Triacetin	0.787 ± 0.163	19
20.	Miglyol 812^{R}	0.783 ± 0.405	20
21.	Isopropyl Palmitate	0.732 ± 0.431	21
22.	Isostearyl Alcohol	0.696 ± 0.430	22
23.	Linoleic Acid	0.576 ± 0.196	23
24.	Imwitor 412^{R}	0.542 ± 0.230	24
25.	Oleic Acid	0.419 ± 0.208	25
26.	PEG 400	0.234 ± 0.204	26

- The epidermal and dermal retention of HC and TAC can be affected rather dramatically by the choice of vehicles. For example, the epidermal and dermal retention ranged **110 and 67 fold**, respectively for HC, and **462 and 84 fold**, respectively for TAC.

- The overall rank order of vehicles for epidermal retention was different than the dermal retention for both HC and TAC. This indicated that the epidermal and dermal retentions were affected differently for the same drug. Also, the magnitudes of the effects noted for HC are quite different than those for TAC.

- The rank order comparisons for epidermal and dermal retentions within different classes of the vehicles studied indicated that four out of seven classes provided different rank orders for HC and six out of seven classes provided different rank orders for TAC.

- The rank order of epidermal to dermal retention ratios for HC was different than for TAC, again emphasizing that vehicles effects vary substantially from drug to drug.

The present studies are important in many respects. The type of <u>in vivo</u> studies done here are useful in selecting vehicle(s) for dermatological formulations. Unfortunately,

these studies also indicate that the state of the art known in this area is insufficient to make prediction on the choice of vehicles. Solubility of drugs in vehicles is not necessarily related to the local drug delivery. Drug delivery scientists have to resort to the experimental approach to select vehicle(s). The observations made in this study, e.g. varying rank orders and ranges, support the contention that selection of vehicles for these two drugs should be based on the experimental determination of **retention** data instead of **flux** data.

6.0 REFERENCES

1. Workshop on the Principles and Criteria in the Development and Optimization of Dermatological Products-Part I. Organized by the Food and Drug Administration (FDA and the American Association of Pharmaceutical Scientist (AAPS), Crystal City, Virginia, March 26-28, 1990.

2. Workshop on the Principles and Criteria in the Development and Optimization of Dermatological Products-Part II. Establishment of Bioavailability and Bioequivalence. Organized by the Food and Drug Administration (FDA and the American Association of Pharmaceutical Scientist (AAPS), Crystal City, Virginia, December 3-5, 1991.

3. Schaefer, H., Zesch, A., and Stuttgen, G in Skin Permeability. Vol II. Publisher: Springer-Verlag, New York, NY. pp 1-2. 1982.

4. Eckert, R. L., " The Structure and Function of the Skin" in Pharmacology of the Skin, Ed. H. Mukhtar, CRC Press, Boca Raton, Ann Arbor, London, 1992, Chapter 1.

5. Boyce, S. T., Epidermis as a Secretory Tissue, J. Invest. Derm., **102**, 8, 1994.

6. Holbrook, K. A., and Wolff, K., The Structure and Development of Skin, in Dermatology in General Medicine, Eds., T. B. Fitzpatrick, A. Z. Eisen, K. Wolff, I. M. Freeburg, and K. F. Ausen, McGraw Hill, New York, 1987. page. 93.

7. Lampe, M. A., Williams, M. L., and Elias, P. M., Human Epidermal Lipids: Characterization and Modulation During Differentiation, J. Lipid Res., **24**, 131, 1983.

8. Wertz, P. W., and Downing, D. T., Glycolipids in Mammalian Epidermis: Localization and Relationship to Cornification, Science, **217**, 1261, 1982.

9. Wertz, P. W., Downing, D. T., Freinkel, R. K., and Traczyk, T. N., Sphingolipids of the Stratum Corneum and Lamellar Granules of Fetal Rat Epidermis, J. Invest. Derm., **83**, 193, 1985.

10. Kleinman, H. K., Klebe, R. J., and Martin, G. R., Role of Collegenous Matrices in the Adhesion and Growth of Cells, J. Cell. Biol., **88**, 473, 1981.

11. Soter, N. A., Mast Cells in Cutaneous Inflammatory Disorders, J. Invest. Derm., **80**, 22s. 1983.

12. Shah, V. P., Behl, C. R., Higuchi, W. I. and Schaefer, H., Principles and Criteria in the Development and Optimization of Topical Therapeutic Products, Pharm. Res., **9**, 1107, 1992.

13. Kasting, G. B., and Robinson, P. J., Can We Assign an Upper Limit to Skin Permeability?, Pharm. Res., **10**, 57, 1993.

14. Reifenrath, W. G., Lee, B., Wilson, D. R., and Spencer, T. S., A Comparison of In Vitro Skin-Penetration Cells, J. Pharm. Sci., **83**, 1229, 1994.

15. Wester, R. C., and Maibach, H. I., Percutaneous Absorption, in Topical Drug Bioavailability, Bioequivalence and Penetration, Eds., V. P. Shah and H. I. Maibach, Plenum Press, New York and London, 1993, Chapter 1, pg. 3.

16. Cleary, G. W., Transdermal Delivery Systems: A Medical Rationale, in Topical Drug Bioavailability, Bioequivalence and Penetration, Eds., V. P. Shah and H. I. Maibach, Plenum Press, New York and London, 1993, Chapter 2, pg. 17.

17. Flynn, G. L., General Introduction and Conceptual Differentiation of Topical and Transdermal Drug Delivery Systems: Differentiation With Respect to Delivery Kinetics, in Topical Drug Bioavailability, Bioequivalence and Penetration, Eds., V. P. Shah and H. I. Maibach, Plenum Press, New York and London, 1993, Chapter 20, pg. 370.

18. Kubota, K., and Maibach, H. I., Significance of Viable Skin Layers in Percutaneous Permeation and Its Implication in Mathematical Models: Theoretical Consideration Based on Parameters for Betamethasone Valerate, J. Pharm. Sci., **83**, 1300, 1994.

19. Kumar, S., Malick, A. W., Meltzer, N. M., Mouskountakis, J. D., and Behl, C. R., In Vitro Skin Permeation and Retention of a Leukotriene Antagonist from Topical Vehicles using Hairless Guinea Pig Model, J. Pharm. Sci., **69**, 631-37, 1992.

20. Reifenrath, W. G., Hawkins, G. S., and Kurtz, M. S., Percutaneous Penetration and Skin Retention of Topically Applied Compounds: An In Vitro-In Vivo Study, J. Pharm. Sci., **80**, 526-30, 1991.

21. Behl, C. R., Char, H., Patel, S. B., Mehta, D., Piemontese, D., Malick, A. W., In Vitro and In Vivo Skin Uptake and Permeation Studies: Critical Considerations and Factors Which Affect Them, in Topical Drug Bioavailability, Bioequivalence, and Penetration, Eds.: V. P. Shah and H. I. Maibach. Plenum Press, New York and London. 1993, Chapter 13.

22. Mehta, D. B., Kumar, S., Patel, S. B., Piemontese, D., Char, H., Meltzer, N. M, Malick, A. W., and Behl, C. R., In Vivo Retention of Ro 24-0238 in the Epidermis and Dermis From Various Topical Vehicles Using Female Hairless Guinea Pig Model. Submitted to J. Pharm. Sci. for publication in 1994.

23. Mehta, D. B., Kumar, S., Patel, S. B., Piemontese, D., Char, H., Meltzer, N. M, Malick, A. W., and Behl, C. R., In Vivo Retention of a Lipoxygenase Inhibitor in the Epidermis and Dermis From Various Topical Vehicles Using Female Hairless Guinea Pig Model. Submitted to J. Pharm. Sci. for publication in 1994.

24. Behl, C. R., Kumar, S, Malick, A. W., Patel, S. B., Char, H. and Piemontese, D., Choice of Membranes for In Vitro Skin Uptake Studies and General Experimental Technics in Methods for Skin Absorption, Eds. B. W. Kemppainen and W. G. Reifenrath, CRC Press, Boca Raton, Ann Arbor, London, 1990, Chapter 1.

25. Behl, C. R., El-Sayed A., and Flynn, G. L., Hydration and Percutaneous Absorption IV. Influence of Hydration on n-Alkanol Permeation Through Rat Skin. Comparison with Hairless and Swiss(Furry) Mice, J. Pharm. Sci., **72**, 79, 1983.

26. Behl, C. R., Barrett M., and Flynn, G. L., Hydration and Percutaneous Absorption II. Influence of Hydration on Water and Alkanol Permeation Through Swiss (Furry) Mouse Skin. Comparison with Hairless Mouse Skin Data, J. Pharm. Sci., **70**, 1212, 1981.

27. Bartec, M. J. and LaBudde, J. A., Percutaneous Absorption In Vitro In: Animal Models in Dermatology, NY, H. I. Maibach, Ed.,Churchill-Livingstone, 1975.

28. Bartec, M. J., LaBudde, J. A., and Maibach, H. I., Skin Permeability In Vivo: Comparison in Rat, Rabbit, Pig and Man, J. Invest. Derm., **58**, 114, 1972.

29. Rougier, A., Dupuis, D., Lotte, C., Roguet R., and Schaefer, H., In Vivo Correlation Between Stratum Corneum Reservoir Function and Percutaneous Absorption, J.

Invest. Derm., **81**, 275, 1983.

30. Walters, M., Dugard, P. H., and Scott, R. C., In Vitro Percutaneous Absorption Studies: A Comparison of Human and Laboratory Species, Human Toxicol., **2**, 561, 1983.

31. Behl, C. R., Harper, N., Pei, J., Kumar, S., Mehta, D., Piemontese, D., Patel, S., Ciccone, G., and Malick, A. W., A General Method for Assessing Skin Permeation Enhancement Mechanisms and Optimization, Drug Permeation Enhancement, Theory and Applications, Ed., Dean S. Hsieh, Marcel Dekker, N.Y., 1994, Chapter 7.

32. Treager, R. T., Factors Affecting Skin Permeability, Molecular Movement, in Theoretical and Experimental Biology, **5**, London, Academic Press, 1966, page 12.

33. Reifenrath, W. G. and Hawkins, G. S., The Weanling Yorkshire Pig as an Animal Model for Measuring Percutaneous Penetration, in Swine in Biomedical Research, Ed., M. E. Tumbleston, 1, Plenum Press, New York, 673, 1980.

34. Kurihara-Bergstrom, T., Woodworth, M., Feisullin, S., and Beall, P., Characteristics of the Yucatan Miniature Pig Skin and Small Intestine for Pharmaceutical Applications, Lab. Anim. Sci., **36**, 396, 1986.

35. Oberle, R. L., Das, H., Wong, S. L., Chan, K. K. H., and Sawchuk, R. J., Pharmacokinetics and Metabolism of Diclofenac Sodkum in Yucatan Miniature Pigs, Pharm. Res., **11**, 698, 1994.

36. Meyer, W., Schwarz, R., and Neurand, K., The Skin of Domestic Mammals as a Model for Human Skin, With Special Reference to the Domestic Pig, in Current Problems in Dermatology, Ed., G. S. Simmons, Z. Paster, M. A. Klingberg, and M. Kaye, **7**, Basel, Karger, 1978.

37. Marzulli, F. N., Brown, D. W. C. and Maibach, H. I., Techniques for Studying Skin Penetration, Toxicol. Appl. Pharmacol. Suppl., **3**, 76-80, 1969.

38. Hunziker, N., Feldmann, R., Maibach, H. I., Animal Models of Percutaneous Penetration: Comparision of Mexican Hairless Dogs and Man, Dermatologica, **156**, 79, 1978.

39. Reifenrath, W. G., Chellquist, E. M., Shipwash, E. A., Jederberg, W. W., and Kruger, G. G., Percutaneous Absorption in Hairless Dog, Weanling pig and Grafted Athymic Nude Mouse: Evaluation of Models for Predicting Skin Penetration in Man, Br. J. Derm., **3**, 27, 123, 1984.

40. Chukwumerijie, O, Nash, R. A., Matias, J., Orentreich, O., Methyl Esters of Alkyl Fatty Acids as Penetration Enhancers for Minoxidil Percutaneous Absorption, Abs. #982, Presented at the 3rd Annual Meeting of the AAPS, Orlando, Florida, 1988.

41. Rischel, W. A., Nathan D., and Hussain, A., Comparison of Skin Permeability for Two Model Substances Using Excised Skin From 5 Mammalian Species, Abs. # 1001, Presented at the 3rd Association of American Pharmaceutical Scientists Meeting, Oct, 1988.

42. Wester, R. C., Noonan, P. K., and Maibach, H. I., Variation in Percutaneous Absorption of Testosterone in the Rhesus Monkey Due to Anatomical Site of Application, Arch. Derm. Res., **267**, 229, 1980.

43. Bhatt, P. P., Rytting, J. H., Topp, E. M., and Higuchi, T., Effect of Azone and Lauryl Alcohol on Transport of Acetaminophen Through Shed Snake Skin, Abs. # 991, Presented at the 3rd Annual AAPS Meeting, Orlando, Florida, 1988.

44. Takahashi, K., Tamagawa, S., Katagi, T., Rytting, J. H., Nishihata, T., and Mizuno, N., Percutaneous Permeation of Basic Compounds Through Shed Snake Skin as a Model Membrane, J. Pharm. Pharmacol., **45**, 882, 1993.

45. Bronaugh, R. L., and Stewart, R. F., Methods for In Vitro Percutaneous Absorption Studies VI: Preparation of the Barrier Layer, J. Pharm. Sci., **75 (5)**, 487, 1986.

46. Bronaugh, R. L. , Stewart, R. F., and Simon, M., Methods for In Vitro Percutaneous Absorption Studies VII:, Use of Excised Human Skin, J. Pharm. Sci., **75 (11)**, 1094, 1986.

47. Campbell, P., Watanabe, T., and Chandrasekaran, S. K., Comparison of Skin Permeability of Scopolamine in Rat, Rabbit and Man, Am. Soc. Exp. Biol., **36**, 639, 1976.

48. Morimoto, Y. , Hatanaka, T., Sugibayashi, K., and Omiya, H., Prediction of Skin Permeability of Drugs: Comparison of Human and Hairless Rat Skin, J. Pharm. Pharmacol. **44**, 534, 1992.

49. Hayashi, R., Sugibayashi, K., and Morimoto, K., Calculation of Skin Permeability Coefficient for Ionized and Unionized Species of Indomethacin, Chem. Pharm. Bull., **40**, 3090, 1992.

50. Kadir, R., Stempler, D., Liron, Z., and Cohen, S., Penetration of Adenosine into Excised Human Skin from

Binary Vehicles: The Enhancement Factor, J. Pharm. Sci., **77 (5)**, 409, 1988.

51. Menczel, E., and Maibach, H. I., In Vitro Human Percutaneous Penetration of Benzyl Alcohol and Testosterone: Epidermal-Dermal Retention, J. Invest. Derm., **54 (5)**, 1970.

52. Busse, M. J., Hunt, P., Lees, K. A., Maggs, P. N. D., and McCarthy, T. M., Release of Betamethasone Derivatives From Ointments-In Vivo and In Vitro Studies, Br. J. Derm., **81** suppl.4, 103, 1969.

53. Fabin B., and Touitou, E., Localization of Lipophilic Molecules Penetrating Rat Skin In Vivo by Quantitative Autoradiography, Int. J. of Pharm., **74**, 59, 1990.

54. Rougier, A., Dupuis, D., Lotte, C., and Roguet, R., The Measurement of the Stratum Corneum Reservoir. A Predictive Method for In Vivo Percutaneous Absorption Studies: Influence of Application Time, J. Invest. Derm., **84**, 66, 1985.

55. Rougier, A., Lotte, C., and Maibach, H. I., The Hairless Rat: A Relevant Animal Model to Predict In Vivo Percutaneous Absorption in Humans, J. Invest. Derm., **88**, 577, 1987.

56. Behl, C. R., and Malick, A. W., Unpublished Data.

57. Okamoto, H., Muta, K., Hashida, M., and Sezaki, H., Percutaneous Penetration of Acyclovir Through Excised Hairless Mouse and Rat Skin: Effect of Vehicle and Percutaneous Penetration Enhancer, Pharm. Res., 64, 1990.

58. Yu, D., Sanders, L. M., Davidson III, G. W. R., Marvin, M. J., and Ling, T., Percutaneous Absorption of Nicardapine and Ketorolac in Rhesus Monkeys, Pharm. Res. **5 (7)**, 457, 1988.

59. Wearly L., and Chien, Y. W., Enhancement of the In Vitro Skin Permeability of Azidothymidine (AZT) via Iontophoresis and Chemical Enhancer, Pharm. Res., **7 (1)**, 34, 1990.

60. Organogenesis, Inc., 83, Rogers St., Cambridge, MA 02142.

61. Advanced Tissue Sciences, 10933, North Torrey Pines Rd., La Jolla, CA 92037-1005.

62. Bell, E., Gay, R., Swiderek, M., Class, T., Paul Kemp., B. S., Green, G., Haimes, H., and Bilbo, P., Use of

Fabricated Living Tissue and Organ Equivalents as Defined Higher Order Systems for the Study of Pharmacologic Responses to Test Substances Presented at the NATO Advanced Research Workshop, Pharmaceutical Application of Cell and Tissue Culture to Drug Transport, Bandol, France, September 4-9, 1989.

63. Fleischmajer, R., Contard, P., Schwartz,E., Douglas MacDonald II, E., Jacobs II, L., and Sakai, L., Elastin-Associated Microfibrils (10 nm) in a Three-Dimensional Fibroblast Culture J. of Invest. Derm., **97**, 639, 1991.

64. Triglia, D., Braa, S. S., Donnelly, T., Kidd, I., and Naughton, G., A Three Dimensional Human Dermal Model Substrate For In Vitro Toxicological Studies, in In Vitro Toxicology: Mechanisms and New Technologies, Ed., Goldberg, A. M., Mary Ann Liebert, New York, 1991, Chapter 8.

65. Landeen, L. K., Zeigler, F. C., Halberstadt, C., Cohen, and Slivka, S. R., Characterization of a Human Dermal Replacement, Wounds, **4**, 167, 1992.

66. Faratash, M., and Ponec, M., Improved Barrier Structure Formation in Air-Exposed Human Keratinocyte Culture Systems, J. Invest. Derm., **102**, 366, 1994.

67. Slivka, S. R., Landeen, L. K., Zeigler, F., Zimber, M., and Bartel, R., Characterization, Barrier Function, and Drug Metabolism of an In Vitro Skin Model, J. Invest. Derm., **100**, 40, 1993.

68. Kumar, S., Char, H., Mehta, D., Patel, S., Ciccone, G., Piemontese, D., Malick, A. W., and Behl, C. R., Permeability of Test Compounds Across Hairless Guinea Pig Skin and Living Skin Equivalent (TestSkin™), Presented at the 6th Annual AAPS Meeting held in Washington, 1991.

69. Shroot, B., Chatelus, A., Civier and Hensby, C. N., A Cellulose Ester Film as an Inert Vehicle for Skin Pharmacological Studies, Br. J. of Derm., III suppl., **27**, 163-7, 1984.

70. DiPolo, R., Sha'afi, R. I., and Solomon, A. K., Transport Parameter in a Porous Cellulose Acetate Membrane, J. Gen. Phys., **55**, 63, 1970.

71. Garret E. R. and Chemburkar, P. B., Evaluation, Control and Prediction of Drug Diffusion Through Polymeric Membranes II. Diffusion of Aminophenone Through Silastic Membranes-A Test of the pH Partition Hypothesis, J.

Pharm. Sci., **57**, 949, 1968.

72. Kumar S., Weinrib, A. B., Dhupar, K. C., Kastrunis, L. M., Mouskountakis, J. D., Behl, C. R., Malick, A. W., Franz, T. J., Evaluation of 14C Ro 10-1670 In Vitro Transport From Various Topical Vehicles Through Hairless Mouse Skin. Presented at the 2nd Annual meeting of the American Association of Pharmaceutical Scientists, Boston, Massachussetts, Pharmaceutics Drug Delivery Abstract PD-517, June 1987.

73. Weinrib, A. B., Dhupar, K. C., Kastrunis, L. M., Kumar, S., Mouskountakis, J. D., Behl, C. R., Malick, A. W. and Franz, T. J., Evaluation of 14C-Ro 15-1570 In Vitro Transport From Various Vehicles Through Hairless Mouse Skin. Presented at the 2nd Annual meeting of the American Association of Pharmaceutical Scientists, Boston, Massachussetts, June 1987. Pharmaceutics Drug Delivery Abstract PD-516.

74. Dhupar, K. C., Kumar S, Weinrib, A. B., Kastrunis, L. M., Mouskountakis, J. D., Behl, C. R., Malick, A. W., Franz, T. J., Evaluation of 14C Iso-Tretinoin In Vitro Transport From Various Topical Vehicles Through Hairless Mouse Skin. Presented at the 2nd Annual meeting of the American Association of Pharmaceutical Scientists, Boston, Massachussetts, June 1987. Pharmaceutics Drug Delivery Abstract PD-518.

75. Yalkowsky, S. H. and Flynn, G. L., Transport of Alkyl Homologs Across Synthetic and Biological Membranes: A New Model for Chain Length-Activity Relationships, J. Pharm. Sci., **62 (2)**, 210, 1973.

76. Flynn, G. L. and Yalkowsky, S. H., Correlation and Prediction of Mass Transport Across Membranes I: Influence of Alkyl Chain Length on Flux-Determining Properties of Barrier and Diffusant, J. Pharm. Sci., Vol 61, **6**, 838, 1972.

77. Hatanaka, T., Inuma, M., Sugibayashi, K., and Morimoto, Y., Prediction of Skin Permeability of Drugs. I. Comparison with Artificial Membrane, Chem. Pharm. Bull., **38**, 3452, 1990.

78. Wuster, D. E and Kramer, S. F., Some Factors Affecting Percutaneous Absorption, J. Pharm. Sci., **50**, 28, 1961.

79. Feldmann, R. J., and Maibach, H. I., Percutaneous Penetration of ^{14}C-Hydrocortisone in Man, Arch. Derm., **94**, 649, 1966.

80. Barry, B. W., Southwell D., and Woodford, R., Optimization of Bioavailability of Topical Steroids: Penetration Enhancers Under Occlusion, J. Invest. Derm., **82(1)**, 49, 1984.

81. Bennett, S. L., Barry, B. W., and Woodford, R., Optimization of Bioavailability of Topical Steroids: Non-occluded Penetration Enhancers Under Thermodynamiuc Control, J. Pharm. Pharmacol., **37**, 298, 1985.

82. Lehman, P. A., and Franz, T. J., Effect of Age and Diet on Stratum Corneum Barrier Function in the Fischer 344 Female Rat, J. Invest. Derm., **100**, 200, 1993.

83. Christopher, E., and Kligman, A. M., Percutaneous Absorption in Aged Skin. In: Montagna, W., Ed., Advances in the Biology of the Skin, Vol. VI. New York: Pergamon Press; pp. 163-175, 1965.

84. Wildnauer, R. H., and Kennedy R., Transepidermal Water Loss of Human New Borns, J. Invest. Derm., **54**, 483, 1970.

85. Behl, C. R., Flynn, G. L., Kurihara, T., Smith, W. M., Bellantone, N. H., Gatmaitan, O. G., Harper, N. J., Higuchi, W. I., and Ho, N. F. H., Age and Anatomical Influences on Alkanol Permeation Of Hairless Mouse, J. Soc. Cosm. Chem., **35**, 237, 1984.

86. Cline, S., Behl, C. R., and Flynn, G. L., Skin Permeability Studies in Hairless and Swiss Furry. Effects of Age and Alkyl Chain Length on <u>In Situ</u> Permeabilities of n-Alkanols. Presented at the 39th APhA meeting , Minneapolis, Minnesota, 1985.

87. Behl, C. R., Bellantone, N. H. and Flynn, G. L., Influence of Age on Percutaneous Absorption of Drug Substances. In: Bronaugh, R. L., Maibach, H. I., Ed., Percutaneous Absorption: Mechanisms, Methodology, Drug Delivery. 1st Ed. New York: Marcel Dekker; 183-212, 1985.

88. Behl, C. R., Mann, K. V. and Bellantone, N. H., Transdermal Delivery of Propranolol. II. Permeation Profiles Through Human Skin. Influences of Hydration, Drug Concentration, Skin Sectioning, pH, Vehicles, Race, Sex and Anatomical Site. Presented at the 131st Annual meeting of the American Pharmaceutical Association, Montreal, Canada, Basic Pharmaceutics Abstract, May 1984.

89. Maibach, H. I., Feldman, R. J., Milby, T. H., Serat, W.

F., Regional Variations in Percutaneous Penetration in Man. Pesticides, Arch. Environ. Health, **23**, 208, 1971.

90. Scheuplein, R. J., Site Variations in Diffusion and Permeability in Physiology and Pathopysiology of the Skin, Ed.: A. Jarret, Vol 5, New York and London, Academic Press. 1978.

91. Scheuplein, R. J., and Blank, I. H., Mechanisms of Percutaneous Absorption. IV. Penetration of Nonelectrolytes (Alcohols) From Aqueous Solutions and Pure Liquids, J. Invest. Derm., **60(5)**, 286, 1973.

92. McKenzie, A. W. and Atkinson, R. M., Topical Activities of Betamethasone Esters in Man, Arch. Derm., **89**, 711, 1964.

93. Poulsen, B. J., Young, E., Coquilla. V., and Katz, M., Effect of Topical Vehicle Composition on the In Vitro Release of Fluocinolone Acetonide and its Acetate Ester, J. Pharm. Sci., **57**, 928, 1968.

94. Ostrenga, J., Haleblian, J., Poulsen, B. J., Ferrell, B., Mueller N., and Sastri S., Vehicle Design for a New Topical Steroid, Fluocinonide, J. Invest. Derm., **56 (5)**, 392, 1971.

95. MacKee, G. M., Sulzberger, M. B., Herrmann, F., and Baer, R. L., Histologic Studies on Percutaneous Penetration With Special Reference to the Effect of Vehicles, J. Invest. Derm., **6**, 43, 1945.

96. Inagi, T., Muramatsu, T., Nagai, H., and Terada, H., Influence of Vehicle Composition on the Penetration of Indomethacin through Guinea-Pig Skin, Chem. Pharm. Bull., **29 (6)**, 1708, 1981.

97. Mollgaard, B. and Hoelgaard. A., Permeation of Estradiol Through the Skin-Effect of Vehicles, Int. J. Pharm., **15**, 185, 1983.

98. Johnson, R., Nusbaum, B. P., Horowitz, S. N. and Frost, P., Transfer of Topically Applied Tetracycline in Various Vehicles Arch. Derm., **119**, 660, 1983.

99. Nicolau, G., Dahlin, D.C., Kohlbrenner, M., Chan, P. S., Ronsberg, M. A., Saunders, T. K., Yacobi, A., and Cervoni, P., Skin Metabolism and Transdermal Absorption of Viprostol, a Synthetic PGE2 Analog, in the Rat: Effect of Vehicle, Skin Pharmacol., **2(1)**, 22, 1989.

100. Koo Lee, C., Uchida, T., Noguchi, E., Kim, N.S., and

Goto, S., Skin Permeation Enhancement of Tegafur by Ethanol/Panasate 800 or Ethanol/Water Binary Vehicle and Combined Effect of Fatty Acids and Fatty Alcohols, J. Pharm. Sci., **82**, 1155, 1993.

101. Yamashita, F., Bando, H., Koyama, Y., Kitagawa, S., Takakura, Y., and Hashida, M., In Vivo and In Vitro Analysis of Skin Penetration Enhancement Based on a Two-Layer Diffusion Model With Polar and Nonpolar Routes in the Stratum Corneum, Pharm. Res., **11**, 185, 1994.

102. Mollgaard B., and Hoelgaard, A., Vehicle Effect on Topical Drug Delivery II. Concurrent Skin Transport of Drugs and Vehicle Components, Acta Pharm. Suec., **20**, 443-50, 1983.

103. Friedlander, S., Dhupar, K. C., Kumar, S., Malick, A. W., and Behl, C. R., Skin Uptake Studies: In Vitro Permeation and Retention of Acid and Salt Forms of Ro 23-3544 from Formulations Using Hairless Guinea Pig Skin and Finite Dose Methodology. Presented at the Eastern Region AAPS, NJ, 1988.

104. Coldman, M. F., Poulsen, B. J., and Higuchi, T., Enhancement of Percutaneous Absorption by the Use of Volatile:Nonvolatile Systems as Vehicles, J. Pharm. Sci., **58 (9)**, 1098, 1969.

105. Baker, H., Experimental Studies on the Influence of Vehicles on Percutaneous Absorption, J. Soc. Cosm. Chem., **20**, 239, 1969.

106. Kaidbey, K.H., Kligman, A.M., Topical Photosensitizers: Influence of Vehicles on Penetration, Arch. Dermat., **110**, 868, 1974.

107. Ziegenmeyer, J., The Influence of the Vehicle on the Absorption and Permeation of Drugs, in Dermal and Transdermal Absorption (Ist International Symposium From 12-14 January, 1981, Munich), Eds: R. Brandau and B. Lippold, WVG mbH Stuttgart, 1982, Chapter 4.

108. Segal, R., and Pisanty, S., Glycerrhizin Gel as a Vehicle for Idoxuridine I. Clinical Investigations, J. Clin. Pharm. Ther., **12(3)**, 165, 1987.

109. Masini, V., Bonte, F., Meybeck, A., and Weipierre, J., Cutaneous Bioavailability in Hairless Rats of Tretinoin in Liposomes or Gel, J. Pharm. Sci., **82**, 17, 1993.

110. Levinson, K. K., Takayama, K., Isowa, K., Okabe, K., and Nagai, T., Formulation Optimization of Indomethacin Gels

Containing a Combination of Three Kinds of Cyclic Monoterpenes as Percutaneous Penetration Enhancers, J. Pharm. Sci., **83**, 1367, 1994.

111. Wahlgren, S., Lindstrom, A.L., and Friberg, S. E., Liquid Crystals as a Potential Ointment Vehicle, J. Pharm. Sci., **73(10)**, 1484, 1984.

112. Krowczynski, I. and Stozek, T., Liposomen als Wirkstofftrager in der percutanen Therapie, Pharmazie, **39**, 627, 1984.

113. Baillie, A.J., Florence, Hume, L.R., Muirhead, G.T., and Rogerson, A., The Preparation and Properties of Niosomes-nonionic Surfactant Vesicles, J. Pharm. Pharmacol., **37**, 863, 1985.

114. Hofland, H. E. J., van der Geest, R., Bodde, H., Junginger, H. E., and Bouwstra, J. A., Estradiol Permeation From Nonionic Surfactant Vesicles Through Human Stratum Corneum, Pharm. Res., **11**, 659, 1994.

115. Nacht, S., and Katz, M., in "Topical Drug Delivery Formulations", Ed., Osborne, D.W., Marcel Dekker, Chapter 15, 299, 1992.

116. Rolland, A., Wagner, N., Chatelus, C., Shroot, B., Site-Specific Drug Delivery to Pilosebaceous Structures Using Polymeric Microspheres, Pharm. Res., **10**, 1389, 1993.

117. Sarkany, I. and Hadgraft, J. W., The Influence of Formulation on Topical Corticosteroid Activity, Br. J. Derm., **81**, **Suppl 4**, 98-102, 1969.

118. Demski, R. E., Demarco, J. D., and Marcus, A. D., An In Vitro Study of the Relative Moisture Occlusive Properties of Several Topical Vehicles and Saran Wrap, J. Invest. Derm., **44**, 361, 1965.

119. Coldman, M. F. and Lockerbie, L., Placebo Ointment Response in the Blanching Test, Br. J. Derm., **85**, 398, 1971.

120. Woodford, R. and Barry, B. W., The Placebo Response to White Soft Paraffin/Propylene Glycol in the Skin Blanching Test, Br. J. Derm., **89**, 53, 1973.

121. Woodford, R. and Barry, B. W., Comparative Bioavailability of Proprietary Hydrophilic Topical Steroid Preparations, J. Pharm. Pharmacol., **Suppl 25**, 123, 1973b.

122. Physicians Desk Reference, 1994.

123. Kligman, A. M., Dimethyl Sulfoxide, Part 2. J. A. M. A., **193**, 923, 1965.

124. Jacob, S. W., and Rosenbaum, E. E., Ann. N. Y. Acad. Sci., **141**, 1, 1967.

125. Schumacher, G. E., Drug Intelligence, **1**, 188, 1967.

126. Jacob, S. W., Rosenbaum, E. E., and Wood, D. C., Dimethyl Sulfoxide, Vol. 1., Basic Concepts, Dekker, New York, 1971.

127. Jacob, S. W., and Herschler, R., Eds., Biological Actions of Dimethyl Sulfoxide, Ann. N. Y. Acad. Sci., 243, 1975.

128. Jacob, S. W., and Wood, D. C., Notes on DMSO Symposium, Curr. Ther. Res., **9**, 299, 1967.

129. Brobyn, R. D., in Biological Actions of Dimethyl Sulfoxide, Eds., Jacob, S. W., and Herschler, R., Ann. N. Y. Acad. Sci., 243, 497, 1975.

130. Sweeney, T. M., Downer, A. M., and Matolsty, A. G., The Effect of Dimethylsulfoxide on the Epidermal Water Barrier, J. of Invest. Derm., **46**, 300, 1966.

131. Allenby, A. C., Fletcher, J, Schock, C., and Tees, T. F. S., The Effect of heat, pH and Organic Solvents on the Electrical Impedance and Permeability of Excised Human Skin, Br. J. Derm., **81**, **suppl. 4**, 31, 1969.

132. Elbaum, S. G., and Laden, K., The Effect of Dimethyl Sulfoxide on Percutaneous Absorption: A Mechanistic Study part I., J. Soc. Cosm. Chem., **19**, 119, 1968.

133. Dugard, P. H., and Embery, G., The Influence of Dimethyl Sulfoxide on the Percutaneous Migration of Potassium Butyl (^{35}S) Sulfate, Potassium Methyl (^{35}S) and Sodium (^{35}S) Sulfate, Br. J. Derm., **81**, **Suppl. 4**, 69, 1969.

134. Embery, G., and Dugard, P. H., The Influence of Dimethyl Sulphoxide on the Percutaneous Migration of Potassium Dodecyl (^{35}S) Sulphate, Br. J. Derm., **81**, **Suppl. 4**, 63, 1969.

135. Chandrasekaran, S. K., Campbell, P., and Micahels, A. S., Effect of DMSO on Drug Permeation Through Human Skin, Am. Inst. Chem. Eng., **23**, 810, 1977.

136. Christie, G. A., and Moore-Robinson, M., Vehicle

Assessment Methodology and Results, Br. J. Derm., **82**, **Suppl. 6**, 93, 1970.

137. Caldwell, I. W., Hall-Smith, S. P., Main, R. A., Ashurst, P. J., Kurton, V., Simpson, W. T., and Williams, G. W., Clinical Evaluation of a New Topical Corticosteroid Beclomethasone, Dipropionate, Br. J. Derm., **80**, 11, 1968.

138. Dillaha, C. J., Jansen, G. T., and Honeycutt, M. W., Q. Bull. Dermatol. Found., 1, New York, 1966.

139. Tissot, J., and Osmundsen, P. E., Influence of Vehicles on the Topical Activity of Fluorometholone, Acta. Derm. Venerol., (Stock.), **46**, 447, 1966.

140. Brode, E., Die Percutane Resorption Von Costicosteroiden II: Die Resorption Von Fluocinolon-acetonid Durch Normale Rattenjaut Und Ihre Beeinflussung, Arzneim. Forsch., **18**, 580, 1968.

141. Barrett, C. W., Hadgraft, J. W., Caron, G. A., and Sarkany, I., The Effect of Particle Size and Vehicle on the Percutaneous Absorption of Fluocinolone Acetonide, Br. J. Derm., **77**, 576, 1965.

142. Portnoy, B., The Effect of Formulation on the Clinical Response to Topical Fluocinolone Acetonide, Br. J. Derm., **77**, 579, 1965.

143. Turi, J. S., Danielson, D., and Woltersom, J. W., Effects of Polyoxypropylene-15 Stearyl Ether and Propylene Glycol on Percutaneous Penetration Rate of Diflorasone Acetate, J. Pharm. Sci., **68**, 275, 1979.

144. Eaglstein, W. H., Weinstein, G. D., and Frost, P., Fluorouracil: Mechanism of Action in Human Skin and Actinic Keratosis, Arch. Derm., **101**, 132, 1970.

145. Whitefield, M. and McKenzie, A. W., A New Formulation of 0.1 % Hydrocortisone Cream With Vasoconstrictor Activity and Clinical Effectiveness, Br. J. Derm., **92**, 585, 1975.

146. Yum, S., Lee, E., Taskovich, L., and Theeuwes, F., Permeation Enhancement With Ethanol: Mechanism of Action Through Skin, in Drug Permeation Enhancement, Ed: D. S. Hsieh, Marcel Dekker, New York, Basle and Hong Kong, 1994, Chapter 8.

147. Laufer, L., DeFazio, J., Lu, Meldrum, D., Eggene, P.,

Sambhi, M., Hershman, J., and Judd, H., Estrogen Replacement Therapy by Transdermal Estradiol Administration, Am. J. Obstet. Gynecol., **146(5)**, 533, 1983.

148. Good, W., Powers, M., and Campbell, P., A new Transdermal Delivery System For Estradiol, J. Cont. Rel., **2**, 89, 1985.

149. Campbell, P., and Chandrasekaran, S. K., U. S. Patent 4,379,454 (Assigned to ALZA Corporation), 1983.

150. Caplan, R., and Southam., M., Transdermal Drug Delivery and its Application to Pain Control, Adv. Pain. Res. Ther., **14**, 233, 1990.

151. Bettley, F. R., The Influence of Detergents and Surfactants on Epidermal Permeability, Br. J. Derm., **77**, 98, 1965.

152. Wood, D. C. F., and Bettley, F. R., Effects of Various Detergents on Human Epidermis, Br. J. Derm., **84**, 320, 1971.

153. Scala, J., McOsker, D. E., Reller, H. H., The Percutaneous Absorption of Ionic Surfactants, J. Invest. Derm., **50(5)**, 371, 1968.

154. Scheuplein, R. J. and Ross, L. W., Effects of Surfactants and Solvents on Permeability of Epidermis, J. Soc. Cosm. Chem., **21**, 853, 1970.

155. Scheuplein, R. J. and Ross, L. W., Mechanisms of Percutaneous Absorption. V. Percutaneous Absorption of Solvent Deposited Solids, J. Invest. Derm., **62**, 353-360, 1974.

156. Ghanem, A. H., Mahmoud, H., Higuchi, W. I., Rohr, U. D., Borsadia, S., Liu, P., Fox, J., and Good, W., The Effects of Ethanol on Transport of B-Estradiol and Other Permeants in Hairless Mouse Skin II. A New Quantitative Approach, J. Contr. Rel., **6**, 75, 1987.

157. Koyama, Y., Bando, H, Yamashita, F., Takakura, Y., Sezaki, H., and Hashida, M., Comparative Analysis of Percutaneous Absorption Enhancement by d-limonene and Oleic Acid Based on a Skin Diffusion Model, Pharm. Res., **11**, 13, 1994.

158. Barry, B. W., Mode of Action of Penetration Enhancers in Human Skin, J. Contr. Rel., **6**, 85-97, 1987.

159. Knutson, K., Potts, R. O., Guzek, D. B., Golden, G. M., Mckie, J. E., Lambert, W. J. and Higuchi, W. I., Macro and Molecular Physical-Chemical Considerations in Understanding Drug Transport in the Stratum Corneum, J. of Cont. Rel., **2**, 67, 1985.

160. Krill, S., Knutson, K., and Higuchi, W. I., Effects of Ethanol on the Stratum Corneum Lipid Domain, Pharm. Res., **5(10)**, S129, 1988.

161. Golden, G., McKie, J., and Potts, R. O., Role of Stratum Corneum Lipid Fluidity in Transdermal Drug Flux, J. Pharm. Sci., **76(1)**, 25, 1987.

162. Seki, T., Sugibayashi, K., and Morimoto, Y., Effects of Solvents on Permeation of Nicardapine HCl Through Hairless Rat Skin, Chem. Pharm. Bull., **35(7)**, 3054, 1987.

163. Berner, B., Masenga, G., Otte, J., Steffens, R., Ethanol:Water Mutually Enhanced Transdermal Therapeutic Systems II: Skin Permeation of Ethanol and Nitroglycerin, J. Pharm. Sci., **78(5)**, 402, 1989.

164. Pershing. L., Lambert, L., and Knutson, K., Pharm. Res., **7(2)**, Mechanism of Ethanol-Enhanced Estradiol Permeation Across Human Skin In Vitro, 170, 1990.

165. Kobayashi, D., Matsuzawa, T., Sugibayashi, K., Morimoto, Y., and Kimura, M., Analysis of the Combined Effect of 1-Menthol and Ethanol as Skin Permeation Enhancers Based on a Two-Layer Skin Model, Pharm. Res., **11**, 96, 1994.

166. Cornwell, P. A., and Barry, B. W., Sesquiterpene Components of Volatile Oils as Skin Penetration Enhancers for the Hydrophilic Permeant 5-Fluorouracil, J. Pharm. Pharmacol., **46**, 261, 1994.

167. Vaidyanathan, R., Rajadhyaksha, V. J., Kim, B. K., Anisko, J. J., in, Transdermal Delivery of Drugs, Eds., A. F. Kaydonius and B. Berner, CRC Press, Boca Raton, FL, 1987, Chapter 5.

168. Touitou, E., Sink Permeation Enhancement by n-Decyl Methyl Sulfoxide: Effect of Solvent Systems and Insights on Mechanisms of Action, Int. J. Pharm., **43**, 1, 1988.

169. Adachi, Y., Hosoya, K., Sugibayashi, K., Morimoto, Y., Duration and Reversibility of the Penetration-Enhancing Effect of Azone^{R}, Chem. Pharm. Bull., **36**, 3702, 1988.

170. Sugibayashi, K., Morimoto, Y., and Nemoto, M., Effect of Several Penetration Enhancers on the Percutaneous Absorption of Indomethacin in Hairless Rats, Chem. Pharm. Bull., **36**, 1519, 1988.

171. Aungst, B. J., Rogers, N. J., and Shefter, E., Enhancement of Naloxone Penetration Through Human Skin In Vitro Using Fatty Acids, Fatty Alcohol, Surfactants, Sulfoxides and Occlusion, Int. J. Pharm., **33**, 225, 1986.

172. Francoeur, M. L., and Potts, R. O., Transdermal Flux-enhancing Pharmaceutical Compositions, U. S. Patent 86-925641, 1986.

173. Tiemessen, H. L. G. M., Bodde, H. E., Mollee, H., Junginger, H. E., A Human Stratum Corneum-silicone Membrane Sandwich to Simulate Drug Transport Under Occlusion, Int. J. Pharm., **53**, 119, 1989.

174. Hori, J., Linewar-Burke Analysis of Skin Penetration Enhancement, J. Controll. Rel., **16**, 263, 1991.

175. Niazi, M. L., Molokhia, A. M., and El-Gorashi, A. S., Effect of Azone^{R} and Other Penetration Enhancers on the Percutaneous Absorption Dihydroergotamine, Int. J. Pharm., **56**, 181, 1989.

176. Sugibayashi, K., Sakonue, C., and Morimoto, Y., Utility of Topical Formulations of Morphine Hydrochloride Containing Azone^{R} and N-Methyl-2-Pyrrolidone, Sel. Cancer. Ther., **5**, 119, 1989.

177. Vasavada, R. C. and Patel, R. A., Investigation of Physicochemical and Formulation Parameters for Transdermal Delivery of Isoproterenol Hydrochloride, Proceedings of Third European Congress of Biochemistry and Pharmacokinetics, **Vol. 1**, 1987, pp. 218.

178. Patel, R. A., and Vasavada, R. C., Transdermal Delivery of Isoproterenol Hydrochloride: An Investigation of Stability, Solubility, Partition Coefficients and Vehicle Effects, Pharm. Res., **5**, 116, 1988.

179. Touitou, E., Transdermal Delivery of Anxiolytics: In Vitro Skin Permeation of Midazolam Maleate and Diazepam, Int. J. Pharm., **33**, 37, 1986.

180. Behl, C. R., Pei, J., and Malick, A. W., Skin Permeation Profiles of Diazepam, Presented at 133rd Annual Meeting Am. Pharm. Assoc., San Fransisco, CA, March 1986, Basic Pharm., Abstr. 2.

181. Kumar, S., Char, H., Patel S. B., Piemontese, D., Malick, A. W., and Behl, C. R., In Vitro Transdermal Delivery of 14C-Clonazepam Across Hairless Guinea Pig Skin, Presented at the 6th Annual AAPS Meeting held in Washington DC, 1991. Abstract # PDD 7316.

182. Schuckler, F., and Lee, G., Measuring the Uptake of Azone into Excised Human Stratum Corneum from Thin Polymer Films, J. Pharm. Pharmacol., **45**, 162, 1993.

183. Bhargava, K., Cutaneous and Percutaneous Absorption of Erythromycin, M.S. Thesis, 1987.

184. Yamamoto, S., Jiang, H., and Kato, R., Stimulation of Hair Growth by Topical Application of FK506, a Potent Immunosuppresive Agent, J. Invest. Derm., **102**, 160, 1994.

185. Boehnlein, J., Sakr, A., Lichtin, J. L., and Bronaugh, R. L., Characterization of Esterase and Alcohol Dehydrogenase Activity in Skin. Metabolism of Retinyl Palmitate to Retinol (Vitamin A) During Percutaneous Absorption, Pharm. Res., **11**, 1155, 1994.

186. Andersen, K. E., Maibach, H. I., and Anjo, M. D., The Guinea Pig: An Animal Model For Human Skin Absorption of Hydrocortisone, Testosterone, and Benzoic Acid?, Br. J. Derm., **102**, 447, 1980.

187. Feldmann, R. J., and Maibach, H. I., Absorption of Some Organic Compounds Through the Skin in Man, J. Invest. Derm., **54**, 399, 1970.

188. Sherertz, E. F., Sloan, K. B., and McTierman, R. G., Effect of Skin Pretreatment With Vehicle Alone or Drug in Vehicle on Flux of a Subsequently Applied Drug: Results of Hairless Mouse Skin and Diffusion Cell Studies, J. Invest. Derm., **89**, 249, 1987.

189. Washitake, M., Anmo, T., Tanaka, I., Arita, T., and Nakano, M., Percutaneous Absorption of Drugs IV: Percutaneous Absorption of Drugs from Oily Vehicles, J. Pharm. Sci., **64**, 397, 1975.

190. Pershing, L. K., In Vitro-In Vivo Correlation of Percutaneous Absorption with Human Skin. AAPS/FDA/AAD/SPS/USEHA Workshop, May 1989, Washington, DC.

191. Yano, T., Higo, N., Fukuda, K., Tsuji, M., Noda, K., and Otagiri, M., Further Evaluation of a New Penetration Enhancer, HPE-101, J. Pharm. Pharmacol., **45**, 775, 1993.

192. Monteiro-Riviere, N. A., Inman, A. O., Riviere, J. E., McNeill, S. C., and Francouer, M. L., Topical Penetration of Piroxicam Is Dependent on the Distribution of the Local Cutaneous Vasculature, Pharm. Res., **10**, 1326, 1993.

193. Tymes, N.W., The Determination of Corticoids and Related Steroid Analogs by High-Performance Liquid Chromatography, J. of Chrom. Sci., **5**, 151, 1977.

194. Kumar, S., Behl, C. R., Patel, S. B., and Malick, A. W., A Simple Microwave Technique for the Separation of Epidermis and Dermis in Skin Uptake Studies, Pharm Res., **6**, 740, 1989.

195. Wiechers, J. W., Absorption, Distribution, Metabolism, and Excretion of the Cutaneous Penetration Enhancer Azone, Ph. D. Thesis, University Centre for Pharmacy, Groningen, The Netherlands, 1989.

APPENDIX A

Table A.1. *In Vivo* Retention of Hydrocortisone From Azone in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 23.2 mg/mL Solution Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	120.4	125.3	142.3	129.3 ± 11.5
Epidermal Wt. (mg)	24.6	39.1	35.1	32.9 ± 7.48
% of Dose Retained	0.671	1.11	1.13	0.970 ± 0.260

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	11.5	10.9	12.2	11.5 ± 0.651
Dermal Wt. (mg)	561.8	533.2	511.8	535.6 ± 25.1
% of Dose Retained	1.46	1.32	1.41	1.40 ± 0.0709

Table A.2. In Vivo Retention of Hydrocortisone From Diacetin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 8.65 mg Solution Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	5.87	8.11	7.54	7.17 ± 1.16
Epidermal Wt. (mg)	55.2	31.7	38.9	41.9 ± 12.0
% of Dose Retained	0.161	0.128	0.146	0.145 ± 0.0165

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.410	1.84	0.700	0.983 ± 0.756
Dermal Wt. (mg)	641.6	341.8	439.7	474.4 ± 152.9
% of Dose Retained	0.130	0.312	0.153	0.198 ± 0.0991

Table A.3. *In Vivo* Retention of Hydrocortisone From Diethylene Glycol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 12.6 mg/mL Solution Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	151.8	236.1	171.2	186.4 ± 44.1
Epidermal Wt. (mg)	41.4	37.5	51.0	43.3 ± 6.95
% of Dose Retained	2.21	3.11	3.07	2.80 ± 0.508

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	11.0	13.6	5.53	10.0 ± 4.12
Dermal Wt. (mg)	370.0	327.3	494.7	397.3 ± 87.0
% of Dose Retained	1.42	1.57	0.962	1.32 ± 0.317

Table A.4. *In Vivo* Retention of Hydrocortisone From Dimethyl Sulfoxide in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 143.42 mg/mL of Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	3782	2878	1867	2843 ± 958.3
Epidermal Wt. (mg)	18.2	43.5	55.0	38.9 ± 18.8
% of Dose Retained	2.50	4.56	3.74	3.60 ± 1.03

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	86.7	37.8	25.9	50.1 ± 32.2
Dermal Wt. (mg)	409.0	650.0	350.0	469.6 ± 158.9
% of Dose Retained	1.29	0.895	0.330	0.838 ± 0.483

Table A.5. *In Vivo* Retention of Hydrocortisone From Ethylene Glycol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 6.20 mg/mL of Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	117.0	138.0	184.9	146.6 ± 34.8
Epidermal Wt. (mg)	31.8	22.4	43.8	32.6 ± 10.7
% of Dose Retained	2.69	2.24	5.86	3.60 ± 1.97

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	5.12	4.09	5.79	5.00 ± 0.85
Dermal Wt. (mg)	180.1	367.8	362.1	303.3 ± 106.8
% of Dose Retained	0.67	1.09	1.52	1.09 ± 0.425

Table A.6. In Vivo Retention of Hydrocortisone From Ethanol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 5.05 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	18.7	26.1	31.4	25.4 ± 6.38
Epidermal Wt. (mg)	47.3	54.6	51.5	51.1 ± 3.66
% of Dose Retained	0.919	1.48	1.68	1.36 ± 0.395

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.306	0.399	0.207	0.304 ± 0.0960
Dermal Wt. (mg)	672.1	779.5	714.0	721.9 ± 54.1
% of Dose Retained	0.214	0.323	0.154	0.230 ± 0.0857

Table A.7. In Vivo Retention of Hydrocortisone From Imwitor 408^{R} in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 4.16 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	65.1	41.9	50.6	52.5 ± 11.7
Epidermal Wt. (mg)	59.8	32.1	63.2	51.7 ± 17.1
% of Dose Retained	4.93	1.70	4.05	3.56 ± 1.67

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	2.62	3.96	3.02	3.20 ± 0.688
Dermal Wt. (mg)	683.8	610.6	591.4	628.6 ± 48.8
% of Dose Retained	2.27	3.06	2.26	2.53 ± 0.459

Table A.8. In Vivo Retention of Hydrocortisone From Imwitor 412^R in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 11.2 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	56.6	51.9	60.3	56.3 ± 4.21
Epidermal Wt. (mg)	76.1	50.5	39.9	55.5 ± 18.6
% of Dose Retained	2.11	1.28	1.18	1.52 ± 0.511

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	4.12	3.89	5.26	4.42 ± 0.734
Dermal Wt. (mg)	538.7	575.5	516.7	543.6 ± 29.7
% of Dose Retained	1.09	1.10	1.33	1.17 ± 0.136

Table A.9. In Vivo Retention of Hydrocortisone from Isopropanol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20μL of 2.23 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	10.1	8.62	8.33	9.02 ± 0.949
Epidermal Wt. (mg)	73.6	60.0	58.9	64.2 ± 8.19
% of Dose Retained	1.62	1.13	1.07	1.27 ± 0.302

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.136	0.0950	0.130	0.120 ± 0.0221
Dermal Wt. (mg)	575.6	531.5	586.4	564.5 ± 29.1
% of Dose Retained	0.172	0.111	0.142	0.142 ± 0.0305

Table A.10. *In Vivo* Retention of Hydrocortisone from Isopropyl Myristate in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.16 mg/mL of Solution Appplied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1.55	1.29	1.72	1.52 ± 0.217
Epidermal Wt. (mg)	41.8	16.5	36.4	31.6 ± 13.3
% of Dose Retained	2.23	0.73	2.16	1.71 ± 0.847

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0590	0.113	0.0657	0.0792 ± 0.0294
Dermal Wt. (mg)	839.4	438.5	582.1	620.0 ± 203.1
% of Dose Retained	1.71	1.71	1.32	1.58 ± 0.225

Table A.11. *In Vivo* Retention of Hydrocortisone from Isopropyl Palmitate in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.09 mg/mL of Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1.13	0.822	1.14	1.03 ± 0.181
Epidermal Wt. (mg)	29.4	22.1	28.9	26.8 ± 4.08
% of Dose Retained	2.05	1.11	2.02	1.73 ± 0.534

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0887	0.0699	0.0694	0.0760 ± 0.0110
Dermal Wt. (mg)	468.6	446.4	524.1	479.7 ± 40.0
% of Dose Retained	2.56	1.92	2.24	2.24 ± 0.320

Table A.12. *In Vivo* Retention of Hydrocortisone from Isostearyl Alcohol in Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.57 mg/mL of Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	5.87	8.11	7.54	7.17 ± 1.16
Epidermal Wt. (mg)	55.2	31.7	39.0	42.0 ± 12.0
% of Dose Retained	3.09	2.45	2.80	2.78 ± 0.320

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.410	1.84	0.700	0.983 ± 0.756
Dermal Wt. (mg)	641.6	341.8	439.7	474.4 ± 152.9
% of Dose Retained	2.5	6.00	2.94	3.82 ± 1.90

Table A.13. *In Vivo* Retention of Hydrocortisone from Linoleic Acid in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 6.11 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	32.6	22.9	17.5	24.3 ± 7.65
Epidermal Wt. (mg)	52.4	35.5	16.9	34.9 ± 17.8
% of Dose Retained	1.48	0.704	0.260	0.815 ± 0.617

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	3.40	3.61	2.02	3.01 ± 0.864
Dermal Wt. (mg)	660.0	603.0	556.0	606.3 ± 52.1
% of Dose Retained	1.94	1.88	0.973	1.60 ± 0.542

Table A.14. *In Vivo* Retention of Hydrocortisone from Monoacetin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 6.11 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	18.2	10.1	11.1	13.1 ± 4.42
Epidermal Wt. in mg	57.9	71.5	83.0	70.8 ± 12.6
% of Dose Retained	0.728	0.497	0.637	0.621 ± 0.116

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1.35	0.460	0.841	0.884 ± 0.447
Dermal Wt. (mg)	502.4	480.8	575.6	519.6 ± 49.7
% of Dose Retained	0.469	0.153	0.334	0.319 ± 0.159

Table A.15. *In Vivo* Retention of Hydrocortisone from Miglyol 812^{R} in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.21 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	5.09	4.45	3.58	4.38 ± 0.760
Epidermal Wt. (mg)	53.5	69.5	47.3	56.8 ± 11.5
% of Dose Retained	3.83	4.35	2.38	3.52 ± 1.02

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.399	0.184	0.222	0.268 ± 0.115
Dermal Wt. (mg)	485.2	583.6	548.3	539.0 ± 49.9
% of Dose Retained	2.73	1.51	1.72	1.99 ± 0.652

Table A.16. *In Vivo* Retention of Hydrocortisone From Miglyol 840^{R} in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.45 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	8.08	11.0	11.0	10.0 ± 1.69
Epidermal Wt. (mg)	48.8	30.1	35.2	38.0 ± 9.67
% of Dose Retained	2.56	2.15	2.52	2.41 ± 0.226

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.849	1.05	0.903	0.934 ± 0.104
Dermal Wt. (mg)	469.8	488.9	552.2	503.6 ± 43.1
% of Dose Retained	2.60	3.33	3.24	3.06 ± 0.398

Table A.17. *In Vivo* Retention of Hydrocortisone From Oleic Acid in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.07 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.456	0.446	0.530	0.477 ± 0.0459
Epidermal Wt. (mg)	50.0	51.9	32.2	44.7 ± 10.9
% of Dose Retained	1.68	1.70	1.26	1.55 ± 0.249

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0439	0.0358	0.0439	0.0412 ± 0.00468
Dermal Wt. (mg)	593.9	633.4	601.7	609.7 ± 20.9
% of Dose Retained	1.92	1.67	1.94	1.84 ± 0.150

Table A.18. *In Vivo* Retention of Hydrocortisone From PEG 200 in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 11.9 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	7.60	7.02	4.05	6.22 ± 1.90
Epidermal Wt. (mg)	38.4	45.1	56.1	46.5 ± 8.93
% of Dose Retained	0.111	0.120	0.0860	0.106 ± 0.0176

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.225	0.299	0.279	0.268 ± 0.0383
Dermal Wt. (mg)	634.5	617.1	566.2	605.9 ± 35.5
% of Dose Retained	0.0540	0.0700	0.0600	0.0613 ± 0.00808

Table A.19. *In Vivo* Retention of Hydrocortisone From PEG 300 in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 8.66 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	8.88	4.76	6.37	6.67 ± 2.08
Epidermal Wt. (mg)	60.7	15.6	38.2	38.2 ± 22.6
% of Dose Retained	0.273	0.0380	0.123	0.145 ± 0.119

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.266	0.271	0.213	0.250 ± 0.0321
Dermal Wt. (mg)	611.8	756.1	621.2	663.0 ± 80.7
% of Dose Retained	0.0820	0.104	0.0670	0.0843 ± 0.0186

Table A.20. In Vivo Retention of Hydrocortisone From PEG 400 in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 6.39 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1.27	1.41	3.46	2.05 ± 1.23
Epidermal Wt. (mg)	47.8	31.3	44.1	41.1 ± 8.66
% of Dose Retained	0.0310	0.0220	0.0770	0.043 ± 0.0295

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.217	0.185	0.183	0.195 ± 0.0191
Dermal Wt. (mg)	483.4	633.1	639.6	585.4 ± 88.4
% of Dose Retained	0.0530	0.0590	0.0590	0.0570 ± 0.00346

Table A.21. *In Vivo* Retention of Hydrocortisone From Propylene Glycol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 6.00 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	187.5	166.1	206.9	186.8 ± 20.4
Epidermal Wt. (mg)	29.0	22.9	24.7	25.5 ± 3.13
% of Dose Retained	5.40	3.78	5.08	4.75 ± 0.856

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	5.13	4.55	2.59	4.09 ± 1.33
Dermal Wt. (mg)	274.7	334.5	208.5	272.6 ± 63.0
% of Dose Retained	1.40	1.51	0.536	1.15 ± 0.533

Table A.22. *In Vivo* Retention of Hydrocortisone From Triacetin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 2.0 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1.88	1.54	1.88	1.77 ± 0.196
Epidermal Wt. (mg)	41.0	31.1	29.2	33.8 ± 6.34
% of Dose Retained	0.165	0.103	0.118	0.129 ± 0.0323

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.177	0.0827	0.111	0.124 ± 0.0484
Dermal Wt. (mg)	502.0	427.8	597.6	509.1 ± 85.1
% of Dose Retained	0.191	0.0760	0.143	0.137 ± 0.0578

Table A.23. *In Vivo* Retention of Hydrocortisone From Tributyrin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.12 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.862	1.01	0.902	0.925 ± 0.0766
Epidermal Wt. (mg)	55.3	44.3	32.7	44.1 ± 11.3
% of Dose Retained	1.92	1.80	1.19	1.63 ± 0.391

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0352	0.0399	0.0338	0.0363 ± 0.00320
Dermal Wt. (mg)	534.5	631.8	420.2	528.8 ± 105.9
% of Dose Retained	0.755	1.01	0.572	0.779 ± 0.220

Table A.24. *In Vivo* Retention of Hydrocortisone From Tricaprylin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 1.07 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug (μg/G)	8.50	14.3	11.3	11.4 ± 2.90
Epidermal Wt. (mg)	30.2	20.5	20.6	23.7 ± 5.51
% of Dose Retained	1.01	1.16	0.915	1.03 ± 0.124

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug (μg/G)	0.596	0.430	0.674	0.567 ± 0.125
Dermal Wt. (mg)	496.4	566.6	473.7	512.2 ± 59.3
% of Dose Retained	1.16	0.958	1.26	1.13 ± 0.154

Table A.25. *In Vivo* Retention of Hydrocortisone From Transcutol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 11.9 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	37.9	54.5	48.4	46.9 ± 8.40
Epidermal Wt. (mg)	26.6	30.9	29.9	29.1 ± 2.25
% of Dose Retained	0.414	0.692	0.594	0.567 ± 0.141

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug (μg/G)	2.70	3.15	2.63	2.83 ± 0.282
Dermal Wt. (mg)	522.1	585.2	545.3	550.9 ± 31.9
% of Dose Retained	0.578	0.756	0.588	0.640 ± 0.100

Table A.26. *In Vivo* Retention of Hydrocortisone From Triolein in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.12 mg/mL Solution Applied over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.422	0.610	0.432	0.488 ± 0.106
Epidermal Wt. (mg)	28.3	24.2	19.3	23.9 ± 4.51
% of Dose Retained	0.661	0.818	0.460	0.646 ± 0.179

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.00925	0.0122	0.00994	0.0105 ± 0.00154
Dermal Wt. (mg)	442.1	385.8	523.9	450.6 ± 69.4
% of Dose Retained	0.226	0.261	0.288	0.258 ± 0.0311

APPENDIX B

Table B.1. *In Vivo* Retention of Triamcinolone Acetonide From Azone in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 23.3 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	115.0	138.2	118.4	123.9 ± 12.5
Epidermal Wt. (mg)	28.8	37.8	36.4	34.3 ± 4.84
% of Dose Retained	0.748	1.18	0.974	0.967 ± 0.216

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	10.2	9.02	8.61	9.28 ± 0.825
Dermal Wt. (mg)	547.6	549.3	504.9	533.9 ± 25.2
% of Dose Retained	1.27	1.12	0.983	1.12 ± 0.144

Table B.2. *In Vivo* Retention of Triamcinolone Acetonide From Diacetin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 7.53 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	2.86	3.35	1.81	2.67 ± 0.787
Epidermal Wt. (mg)	45.2	42.8	35.6	41.2 ± 5.00
% of Dose Retained	0.0740	0.0820	0.0370	0.0643 ± 0.0240

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.148	0.197	0.147	0.164 ± 0.0286
Dermal Wt. (mg)	560.2	495.0	348.3	467.8 ± 108.5
% of Dose Retained	0.0470	0.0560	0.0290	0.0440 ± 0.0137

Table B.3. *In Vivo* Retention of Triamcinolone Acetonide From Diethylene Glycol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 7.93 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	30.6	34.8	39.4	34.9 ± 4.40
Dermal Wt. (mg)	62.5	46.9	26.7	45.4 ± 17.9
% of Dose Retained	1.07	0.912	0.588	0.857 ± 0.246

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.664	1.21	0.679	0.851 ± 0.311
Dermal Wt. (mg)	467.9	385.1	286.6	379.9 ± 90.8
% of Dose Retained	0.174	0.262	0.109	0.182 ± 0.0767

Table B.4. *In Vivo* Retention of Triamcinolone Acetonide From Dimethyl Sulfoxide in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 160.8 mg/mL solution applied over 10^2 cm.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1530	1446	931.0	1302 ± 324.3
Epidermal Wt. (mg)	56.8	45.2	52.7	51.6 ± 5.88
% of Dose Retained	2.86	2.15	1.62	2.21 ± 0.622

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	34.0	14.5	21.5	24.3 ± 9.88
Dermal Wt. (mg)	557.9	875.0	775.0	736.0 ± 162.1
% of Dose Retained	0.625	0.419	0.530	0.525 ± 0.103

Table B.5. *In Vivo* Retention of Triamcinolone Acetonide From Ethylene Glycol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 2.32 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	52.6	57.2	48.0	52.6 ± 4.60
Epidermal Wt. (mg)	51.1	25.5	55.6	44.1 ± 16.2
% of Dose Retained	5.22	2.83	5.19	4.41 ± 1.37

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	3.09	0.619	3.52	2.41 ± 1.57
Dermal Wt. (mg)	369.0	299.7	331.1	333.3 ± 34.7
% of Dose Retained	2.21	0.360	2.27	1.61 ± 1.09

Table B.6. In Vivo Retention of Triamcinolone Acetonide From Ethanol in the Epidermis and Dermis of Hairless Rat Skin.Dose Applied = 20 μL of 11.6 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	39.6	46.5	22.2	36.1 ± 12.5
Epidermal Wt. (mg)	56.8	36.5	47.3	46.9 ± 10.2
% of Dose Retained	1.02	0.77	0.475	0.755 ± 0.273

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1.11	1.40	0.700	1.07 ± 0.352
Dermal Wt. in (mg)	756.0	551.5	638.7	648.7 ± 102.6
% of Dose Retained	0.380	0.349	0.203	0.311 ± 0.0945

Table B.7. *In Vivo* Retention of Triamcinolone Acetonide From Imwitor 408^{R} in the Epidermis and Dermis of Hairless Rat Skin Layers. Dose Applied = 20 μL of 3.95 mg/mL solution applied over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	29.8	31.4	34.7	32.0 ± 2.50
Epidermal Wt. (mg)	29.7	63.9	36.8	43.5 ± 18.0
% of Dose Retained	1.18	2.68	1.70	1.85 ± 0.762

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	3.06	2.76	4.03	3.28 ± 0.664
Dermal Wt. (mg)	545.7	531.6	612.5	563.3 ± 43.2
% of Dose Retained	2.23	1.96	3.30	2.50 ± 0.709

Table B.8. *In Vivo* Retention of Triamcinolone Acetonide From Imwitor 412^{R} in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.97 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	5.04	5.12	6.5	5.55 ± 0.821
Epidermal Wt. (mg)	28.3	25.0	38.1	30.5 ± 6.81
% of Dose Retained	0.804	0.721	1.40	0.975 ± 0.370

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.550	0.690	0.570	0.603 ± 0.0757
Dermal Wt. (mg)	517.5	552.5	525.4	531.8 ± 18.4
% of Dose Retained	1.61	2.14	1.77	1.84 ± 0.272

Table B.9. *In Vivo* Retention of Triamcinolone Acetonide From Isopropanol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 1.7 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	6.78	5.06	1.96	4.60 ± 2.44
Epidermal Wt. (mg)	61.0	58.9	73.6	64.5 ± 7.95
% of Dose Retained	1.28	0.926	0.449	0.885 ± 0.417

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0680	0.0860	0.0740	0.0760 ± 0.00917
Dermal Wt. (mg)	671.1	790.3	694.0	718.5 ± 63.3
% of Dose Retained	0.143	0.212	0.160	0.172 ± 0.0359

Table B.10. *In Vivo* Retention of Triamcinolone Acetonide From Isopropyl Myristate in the Epidermis and Dermis of Hairless Rat Skin Layers. Dose Applied = 20 μL of 0.095 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.843	0.750	0.730	0.774 ± 0.0603
Epidermal Wt. (mg)	25.2	45.6	52.7	41.2 ± 14.3
% of Dose Retained	1.25	2.01	2.26	1.84 ± 0.526

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0560	0.0640	0.0620	0.0607 ± 0.00416
Dermal Wt. (mg)	526.1	536.0	677.8	580.0 ± 84.9
% of Dose Retained	1.75	2.01	1.90	1.89 ± 0.13

Table B.11. *In Vivo* Retention of Triamcinolone Acetonide From Isopropyl Palmitate in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.07 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.754	0.590	0.740	0.696 ± 0.0926
Epidermal Wt. (mg)	28.4	38.4	37.1	34.6 ± 5.44
% of Dose Retained	1.73	1.82	2.20	1.92 ± 0.249

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.170	0.0460	0.0670	0.0943 ± 0.0663
Dermal Wt. (mg)	505.4	460.4	474.7	480.2 ± 23.0
% of Dose Retained	6.90	1.68	2.54	3.71 ± 2.80

Table B.12. In Vivo Retention of Triamcinolone Acetonide From Isostearyl Alcohol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.6 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1.42	1.68	1.27	1.46 ± 0.207
Epidermal Wt. (mg)	27.3	24.3	54.8	35.5 ± 16.8
% of Dose Retained	0.352	0.371	0.632	0.452 ± 0.156

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.126	0.185	0.109	0.140 ± 0.0399
Dermal Wt. (mg)	609.3	558.3	531.1	566.2 ± 39.7
% of Dose Retained	0.698	0.938	0.527	0.721 ± 0.206

Table B.13. *In Vivo* Retention of Triamcinolone Acetonide From Linoleic Acid in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.135 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.522	0.906	0.678	0.702 ± 0.193
Epidermal Wt. (mg)	44.9	22.7	36.4	34.7 ± 11.2
% of Dose Retained	0.919	0.806	0.969	0.898 ± 0.0835

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0510	0.0820	0.0750	0.069 ± 0.0163
Dermal Wt. (mg)	586.5	632.9	598.0	605.8 ± 24.2
% of Dose Retained	1.17	2.04	1.76	1.66 ± 0.441

Table B.14. *In Vivo* Retention of Triamcinolone Acetonide From Monoacetin in the Epidermis of Hairless Rat Skin. Dose Applied = 20 μL of 8.34 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	22.6	16.3	37.7	25.5 ± 11.0
Epidermal Wt. (mg)	43.5	24.1	65.0	44.2 ± 20.5
% of Dose Retained	0.493	0.197	1.23	0.640 ± 0.532

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.916	0.811	0.467	0.731 ± 0.235
Dermal Wt. (mg)	633.5	413.5	635.9	561.0 ± 127.7
% of Dose Retained	0.292	0.168	0.149	0.203 ± 0.0777

Table B.15. *In Vivo* Distribution of Triamcinolone Acetonide From Miglyol 812^{R} in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.25 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	2.47	3.75	2.77	3.00 ± 0.669
Epidermal Wt. (mg)	41.4	27.8	22.3	30.5 ± 9.83
% of Dose Retained	1.23	1.25	0.718	1.07 ± 0.302

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.20	0.19	0.32	0.237 ± 0.0723
Dermal Wt. (mg)	524.0	521.0	589.1	544.7 ± 38.5
% of Dose Retained	1.28	1.17	2.24	1.56 ± 0.589

Table B.16. *In Vivo* Retention of Triamcinolone Acetonide From Miglyol 840^{R} in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.35 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	6.10	5.40	5.26	5.59 ± 0.450
Epidermal Wt. (mg)	46.3	45.2	62.9	51.5 ± 9.92
% of Dose Retained	2.39	2.10	2.84	2.44 ± 0.373

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.580	0.530	0.630	0.580 ± 0.0500
Dermal Wt. (mg)	651.7	598.7	551.3	600.6 ± 50.2
% of Dose Retained	3.23	2.72	2.99	2.98 ± 0.255

Table B.17. In Vivo Retention of Triamcinolone Acetonide From Oleic Acid in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.095 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.539	0.302	0.463	0.435 ± 0.121
Epidermal Wt. (mg)	17.2	53.1	21.7	30.7 ± 19.6
% of Dose Retained	0.537	0.928	0.581	0.682 ± 0.214

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0660	0.0400	0.0360	0.0473 ± 0.0163
Dermal Wt. (mg)	613.6	629.3	724.0	655.6 ± 59.7
% of Dose Retained	2.36	1.45	1.49	1.77 ± 0.514

Table B.18. In Vivo Retention of Triamcinolone Acetonide From PEG 200 in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	8.05	3.27	6.70	6.01 ± 2.46
Epidermal Wt. (mg)	26.3	42.1	46.8	38.4 ± 10.7
% of Dose Retained	0.106	0.0690	0.156	0.110 ± 0.0437

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.310	0.210	0.160	0.227 ± 0.0764
Dermal Wt. (mg)	766.8	499.7	519.8	595.4 ± 148.7
% of Dose Retained	0.119	0.0520	0.0410	0.0707 ± 0.0422

Table B.19. *In Vivo* Retention of Triamcinolone Acetonide From PEG 300 in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 7.75 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	1.13	2.86	1.90	1.96 ± 0.867
Epidermal Wt. (mg)	38.7	32.2	46.0	40.0 ± 6.90
% of Dose Retained	0.0250	0.0520	0.0500	0.0423 ± 0.0150

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.140	0.170	0.140	0.150 ± 0.0173
Dermal Wt. (mg)	702.5	429.7	609.3	580.5 ± 138.7
% of Dose Retained	0.0560	0.0400	0.0430	0.0463 ± 0.00850

Table B.20. *In Vivo* Retention of Triamcinolone Acetonide From PEG 400 in the Epidermis and Dermis Hairless Rat Skin. Dose Applied = 20 μL of 6.55 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.190	0.340	0.800	0.443 ± 0.318
Epidermal Wt. (mg)	55.7	26.8	49.1	43.9 ± 15.1
% of Dose Retained	0.00600	0.00500	0.0220	0.011 ± 0.00954

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.139	0.128	0.130	0.132 ± 0.00586
Dermal Wt. (mg)	665.1	590.5	652.0	635.9 ± 39.8
% of Dose Retained	0.0520	0.0430	0.0470	0.0473 ± 0.00451

Table B.21. In Vivo Retention of Triamcinolone Acetonide From Propylene Glycol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 6.06 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	153.5	197.6	138.1	163.1 ± 30.9
Epidermal Wt. (mg)	24.3	26.7	48.9	33.3 ± 13.6
% of Dose Retained	3.61	5.11	6.54	5.09 ± 1.47

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	3.24	0.962	2.06	2.09 ± 1.14
Dermal Wt. (mg)	341.7	308.6	475.5	375.3 ± 88.4
% of Dose Retained	1.07	0.287	0.950	0.769 ± 0.422

Table B.22. *In Vivo* Retention of Triamcinolone Acetonide From Triacetin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 1.9 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	2.69	1.66	1.27	1.87 ± 0.734
Epidermal Wt. in mg	38.0	33.1	39.1	36.7 ± 3.19
% of Dose Retained	0.233	0.125	0.113	0.157 ± 0.0661

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.149	0.156	0.109	0.138 ± 0.0254
Dermal Wt. in mg	717.8	553.8	590.8	620.8 ± 86.0
% of Dose Retained	0.243	0.197	0.147	0.196 ± 0.0480

Table B.23. *In Vivo* Retention of Triamcinolone Acetonide From Tributyrin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.095 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.838	0.727	0.901	0.822 ± 0.0881
Epidermal Wt. (mg)	47.5	40.2	26.2	38.0 ± 10.8
% of Dose Retained	2.12	1.55	1.25	1.64 ± 0.442

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0530	0.0550	0.0380	0.0487 ± 0.00929
Dermal Wt. (mg)	379.5	412.5	495.4	429.1 ± 59.7
% of Dose Retained	1.07	1.20	0.988	1.09 ± 0.107

Table B.24. *In Vivo* Retention of Triamcinolone Acetonide From Tricaprylin in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.30 mg/mL Solution Applied Over 10^2 cm.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	3.56	2.73	2.13	2.81 ± 0.718
Epidermal Wt. (mg)	25.9	35.4	48.7	36.7 ± 10.5
% of Dermis Retained	1.53	1.60	1.72	1.62 ± 0.0961

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0800	0.0850	0.0790	0.0813 ± 0.00321
Dermal Wt. (mg)	535.0	560.8	471.9	522.6 ± 45.7
% of Dose Retained	0.686	0.791	0.620	0.699 ± 0.0862

Table B.25. In Vivo Retention of Triamcinolone Acetonide From Transcutol in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 6.63 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	42.0	53.3	37.7	44.3 ± 8.06
Epidermal Wt. (mg)	26.7	26.1	48.9	33.9 ± 13.0
% of Dose Retained	0.822	1.02	1.35	1.06 ± 0.267

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	2.10	1.66	0.883	1.55 ± 0.616
Dermal Wt. (mg)	479.7	586.7	610.8	559.1 ± 69.8
% of Dose Retained	0.736	0.712	0.398	0.614 ± 0.190

Table B.26. *In Vivo* Retention of Triamcinolone Acetonide From Triolein in the Epidermis and Dermis of Hairless Rat Skin. Dose Applied = 20 μL of 0.11 mg/mL Solution Applied Over 10 cm^2.

Epidermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.799	1.70	1.45	1.32 ± 0.465
Epidermal Wt. (mg)	29.0	22.4	53.8	35.1 ± 16.6
% of Dose Retained	1.09	1.80	3.65	2.18 ± 1.32

Dermis

Parameters	Animal 1	Animal 2	Animal 3	Average ± S.D.
Drug Retained (μg/G)	0.0220	0.0730	0.0460	0.0470 ± 0.0255
Dermal Wt. (mg)	501.6	258.5	475.0	411.7 ± 133.3
% of Dose Retained	0.51	0.886	1.03	0.809 ± 0.268

7. BIBLIOGRAPHY

Adachi, Y., Hosoya, K., Sugibayashi, K., Morimoto, Y., Duration and Reversibility of the Penetration-Enhancing Effect of Azone[R], Chemical and Pharmaceutical Bulletin, **36**, 3702, 1988.

Advanced Tissue Sciences, 10933, North Torrey Pines Rd., La Jolla, CA 92037-1005.

Allenby, A. C., Fletcher, J, Schock, C., and Tees, T. F. S., The Effect of heat, pH and Organic Solvents on the Electrical Impedance and Permeability of Excised Human Skin, British Journal of Dermatology, **81**, **suppl. 4**, 31, 1969.

Andersen, K. E., Maibach, H. I., and Anjo, M. D., The Guinea Pig: An Animal Model For Human Skin Absorption of Hydrocortisone, Testosterone, and Benzoic Acid?, British Journal of Dermatology, **102**, 447, 1980.

Aungst, B. J., Rogers, N. J., and Shefter, E., Enhancement of Naloxone Penetration Through Human Skin In Vitro Using Fatty Acids, Fatty Alcohol, Surfactants, Sulfoxides and Occlusion, International Journal of Pharmaceutics, **33**, 225, 1986.

Baillie, A.J., Florence, Hume, L.R., Muirhead, G.T., and Rogerson, A., The Preparation and Properties of Niosomes-nonionic Surfactant Vesicles, Journal of Pharmacy and Pharmacology, **37**, 863, 1985.

Baker, H., Experimental Studies on the Influence of Vehicles on Percutaneous Absorption, Journal of Society of Cosmetic Chemists, **20**, 239, 1969.

Barrett, C. W., Hadgraft, J. W., Caron, G. A., and Sarkany, I., The Effect of Particle Size and Vehicle on the Percutaneous Absorption of Fluocinolone Acetonide, British Journal of Dermatology, **77**, 576, 1965.

Barry, B. W., Mode of Action of Penetration Enhancers in Human Skin, Journal of Controlled Release, **6**, 85-97, 1987.

Barry, B. W., Southwell D., and Woodford, R., Optimization of Bioavailability of Topical Steroids: Penetration Enhancers Under Occlusion, Journal of Investigative Dermatology, **82(1)**, 49, 1984.

Bartec, M. J., LaBudde, J. A., and Maibach, H. I., Skin Permeability In Vivo: Comparison in Rat, Rabbit, Pig and Man, Journal of Investigative Dermatology, **58**, 114, 1972.

Bartec, M. J. and LaBudde, J. A., Percutaneous Absorption

In Vitro In: **Animal Models in Dermatology**, NY, H. I. Maibach, Ed.,Churchill-Livingstone, 1975.

Behl, C. R., Kumar, S, Malick, A. W., Patel, S. B., Char, H. and Piemontese, D., Choice of Membranes for In Vitro Skin Uptake Studies and General Experimental Technics in **Methods for Skin Absorption**, Eds. B. W. Kemppainen and W. G. Reifenrath, CRC Press, Boca Raton, Ann Arbor, London, 1990, Chapter 1.

Behl, C. R., El-Sayed A., and Flynn, G. L., Hydration and Percutaneous Absorption IV. Influence of Hydration on n-Alkanol Permeation Through Rat Skin. Comparison with Hairless and Swiss(Furry) Mice, Journal of Pharmaceutical Sciences, **72**, 79, 1983.

Behl, C. R., Barrett M., and Flynn, G. L., Hydration and Percutaneous Absorption II. Influence of Hydration on Water and Alkanol Permeation Through Swiss (Furry) Mouse Skin. Comparison with Hairless Mouse Skin Data, Journal of Pharmaceutical Sciences, **70**, 1212, 1981.

Behl, C. R., Pei, J., and Malick, A. W., Skin Permeation Profiles of Diazepam, Presented at 133rd Annual Meeting of the American Pharmaceutical Association, San Fransisco, CA, March 1986, Basic Pharm., Abstr. 2.

Behl, C. R., Bellantone, N. H. and Flynn, G. L., Influence of Age on Percutaneous Absorption of Drug Substances in **Percutaneous Absorption: Mechanisms, Methodology, Drug Delivery**, Bronaugh, R. L., Maibach, H. I., Ed., 1st Ed. New York: Marcel Dekker; 183-212, 1985.

Behl, C. R., Mann, K. V. and Bellantone, N. H., Transdermal Delivery of Propranolol. II. Permeation Profiles Through Human Skin. Influences of Hydration, Drug Concentration, Skin Sectioning, pH, Vehicles, Race, Sex and Anatomical Site. Presented at the 131st Annual meeting of the American Pharmaceutical Association, Montreal, Canada, Basic Pharmaceutics Abstract, May 1984.

Berner, B., Masenga, G., Otte, J., Steffens, R., Ethanol:Water Mutually Enhanced Transdermal Therapeutic Systems II: Skin Permeation of Ethanol and Nitroglycerin, Journal of Pharmaceutical Sciences, **78(5)**, 402, 1989.

Behl, C. R., Flynn, G. L., Kurihara, T., Smith, W. M., Bellantone, N. H., Gatmaitan, O. G., Harper, N. J., Higuchi, W. I., and Ho, N. F. H., Age and Anatomical Influences on Alkanol Permeation Of Hairless Mouse, Journal of the Society of Cosmetic Chemists, **35**, 237, 1984.

Behl, C. R., and Malick, A. W., Unpublished Data.

Behl, C. R., Char, H., Patel, S. B., Mehta, D., Piemontese, D., Malick, A. W., In Vitro and In Vivo Skin Uptake and Permeation Studies: Critical Considerations and Factors Which Affect Them, in **Topical Drug Bioavailability, Bioequivalence, and Penetration**, Eds.: V. P. Shah and H. I. Maibach. Plenum Press, New York and London. 1993, Chapter 13.

Behl, C. R., Harper, N., Pei, J., Kumar, S., Mehta, D., Piemontese, D., Patel, S., Ciccone, G., and Malick, A. W., A General Method for Assessing Skin Permeation Enhancement Mechanisms and Optimization in **Drug Permeation Enhancement, Theory and Applications**, Ed., Dean S. Hsieh, Marcel Dekker, N.Y., 1994, Chapter 7.

Bell, E., Gay, R., Swiderek, M., Class, T., Paul Kemp., B. S., Green, G., Haimes, H., and Bilbo, P., Use of Fabricated Living Tissue and Organ Equivalents as Defined Higher Order Systems for the Study of Pharmacologic Responses to Test Substances Presented at the NATO Advanced Research Workshop, Pharmaceutical Application of Cell and Tissue Culture to Drug Transport, Bandol, France, September 4-9, 1989.

Bennett, S. L., Barry, B. W., and Woodford, R., Optimization of Bioavailability of Topical Steroids: Non-occluded Penetration Enhancers Under Thermodynamiuc Control, Journal of Pharmacy and Pharmacology, **37**, 298, 1985.

Bettley, F. R., The Influence of Detergents and Surfactants on Epidermal Permeability, British Journal of Dermatology, **77**, 98, 1965.

Bhargava, K., Cutaneous and Percutaneous Absorption of Erythromycin, M.S. Thesis, 1987.

Bhatt, P. P., Rytting, J. H., Topp, E. M., and Higuchi, T., Effect of Azone and Lauryl Alcohol on Transport of Acetaminophen Through Shed Snake Skin, Abstract # 991, Presented at the 3rd Annual American Association of Pharmaceutical Scientists Meeting, Orlando, Florida, 1988.

Boehnlein, J., Sakr, A., Lichtin, J. L., and Bronaugh, R. L., Characterization of Esterase and Alcohol Dehydrogenase Activity in Skin. Metabolism of Retinyl Palmitate to Retinol (Vitamin A) During Percutaneous Absorption, Pharmaceutical Research, **11**, 1155, 1994.

Boyce, S. T., Epidermis as a Secretory Tissue, Journal of Investigative Dermatology, **102**, 8, 1994.

Brobyn, R. D., in Biological Actions of Dimethyl Sulfoxide, Eds., Jacob, S. W., and Herschler, R., Annals of New York

Academy of Sciences, **243**, 497, 1975.

Brode, E., Die Percutane Resorption Von Costicosteroiden II: Die Resorption Von Fluocinolon-acetonid Durch Normale Rattenjaut Und Ihre Beeinflussung, Arzneimittal Forsch, **18**, 580, 1968.

Bronaugh, R. L., and Stewart, R. F., Methods for In Vitro Percutaneous Absorption Studies VI: Preparation of the Barrier Layer, Journal of Pharmaceutical Sciences, **75 (5)**, 487, 1986.

Bronaugh, R. L. , Stewart, R. F., and Simon, M., Methods for In Vitro Percutaneous Absorption Studies VII:, Use of Excised Human Skin, Journal of Pharmaceutical Sciences, **75 (11)**, 1094, 1986.

Busse, M. J., Hunt, P., Lees, K. A., Maggs, P. N. D., and McCarthy, T. M., Release of Betamethasone Derivatives From Ointments-In Vivo and In Vitro Studies, British Journal of Dermatology, **81** suppl.4, 103, 1969.

Caldwell, I. W., Hall-Smith, S. P., Main, R. A., Ashurst, P. J., Kurton, V., Simpson, W. T., and Williams, G. W., Clinical Evaluation of a New Topical Corticosteroid Beclomethasone, Dipropionate, British Journal of Dermatology, **80**, 11, 1968.

Campbell, P., and Chandrasekaran, S. K., U. S. Patent 4,379,454 (Assigned to ALZA Corporation), 1983.

Campbell, P., Watanabe, T., and Chandrasekaran, S. K., Comparison of Skin Permeability of Scopolamine in Rat, Rabbit and Man, American Society of Experimental Biology, **36**, 639, 1976.

Caplan, R., and Southam., M., Transdermal Drug Delivery and its Application to Pain Control, Advanced Pain Research Therapeutics, **14**, 233, 1990.

Chandrasekaran, S. K., Campbell, P., and Micahels, A. S., American Institute of Chemical Engineering, **23**, 810, 1977.

Christie, G. A., and Moore-Robinson, M., Vehicle Assessment Methodology and Results, British Journal of Dermatology, **82**, **Suppl. 6**, 93, 1970.

Christopher, E., and Kligman, A. M., Percutaneous Absorption in Aged Skin. In: Montagna, W., Ed., Advances in the Biology of the Skin, Vol. VI. New York: Pergamon Press; pp. 163, 1965.

Chukwumerijie, O, Nash, R. A., Matias, J., Orentreich, O., Methyl Esters of Alkyl Fatty Acids as Penetration Enhancers

for Minoxidil Percutaneous Absorption, Abstract #982, Presented at the 3rd Annual Meeting of the American Association of Pharmaceutical Scientists, Orlando, Florida, 1988.

Cleary, G. W., Transdermal Delivery Systems: A Medical Rationale, in **Topical Drug Bioavailability, Bioequivalence and Penetration**, Eds., V. P. Shah and H. I. Maibach, Plenum Press, New York and London, 1993, Chapter 2, pg. 17.

Cline, S., Behl, C. R., and Flynn, G. L., Skin Permeability Studies in Hairless and Swiss Furry. Effects of Age and Alkyl Chain Length on In Situ Permeabilities of n-Alkanols. Presented at the 39th American Pharmaceutical Association meeting , Minneapolis, Minnesota, 1985.

Coldman, M. F. and Lockerbie, L., Placebo Ointment Response in the Blanching Test, British Journal of Dermatology, **85**, 398, 1971.

Coldman, M. F., Poulsen, B. J., and Higuchi, T., Enhancement of Percutaneous Absorption by the Use of Volatile:Nonvolatile Systems as Vehicles, Journal of Pharmaceutical Sciences, **58 (9)**, 1098, 1969.

Cornwell, P. A., and Barry, B. W., Sesquiterpene Components of Volatile Oils as Skin Penetration Enhancers for the Hydrophilic Permeant 5-Fluorouracil, Journal of Pharmacy and Pharmacology, **46**, 261, 1994.

Demski, R. E., Demarco, J. D., and Marcus, A. D., An In Vitro Study of the Relative Moisture Occlusive Properties of Several Topical Vehicles and Saran Wrap, Journal of Investigative Dermatology, **44**, 361, 1965.

Dhupar, K. C., Kumar S, Weinrib, A. B., Kastrunis, L. M., Mouskountakis, J. D., Behl, C. R., Malick, A. W., Franz, T. J., Evaluation of 14C Iso-Tretinoin In Vitro Transport From Various Topical Vehicles Through Hairless Mouse Skin. Presented at the 2nd Annual meeting of the American Association of Pharmaceutical Scientists, Boston, Massachussetts, June 1987. Pharmaceutics Drug Delivery Abstract PD-518.

Dhupar, K. C., Kumar S, Weinrib, A. B., Kastrunis, L. M., Mouskountakis, J. D., Behl, C. R., Malick, A. W., Franz, T. J., Evaluation of 14C Iso-Tretinoin In Vitro Transport From Various Topical Vehicles Through Hairless Mouse Skin. Presented at the 2nd Annual meeting of the American Association of Pharmaceutical Scientists, Boston,

Massachussetts, June 1987. Pharmaceutics Drug Delivery Abstract PD-518.

Dillaha, C. J., Jansen, G. T., and Honeycutt, M. W., Quarterly Bulletin of Dermatology Foundations, 1, New York, 1966.

DiPolo, R., Sha'afi, R. I., and Solomon, A. K., Transport Parameter in a Porous Cellulose Acetate Membrane, Journal of General Physiology, **55**, 63, 1970.

Dugard, P. H., and Embery, G., The Influence of Dimethyl Sulfoxide on the Percutaneous Migration of Potassium Butyl (^{35}S) Sulfate, Potassium Methyl (^{35}S) and Sodium (^{35}S) Sulfate, British Journal of Dermatology, **81**, **Suppl. 4**, 69, 1969.

Eaglstein, W. H., Weinstein, G. D., and Frost, P., Fluorouracil: Mechanism of Action in Human Skin and Actinic Keratosis, Archives of Dermatology, **101**, 132, 1970.

Eckert, R. L., " The Structure and Function of the Skin" in **Pharmacology of the Skin**, Ed. H. Mukhtar, CRC Press, Boca Raton, Ann Arbor, London, 1992, Chapter 1.

Elbaum, S. G., and Laden, K., The Effect of Dimethyl Sulfoxide on Percutaneous Absorption: A Mechanistic Study part I., Journal of the Society of Cosmetic Chemists, **19**, 119, 1968.

Embery, G., and Dugard, P. H., The Influence of Dimethyl Sulphoxide on the Percutaneous Migration of Potassium Dodecyl (^{35}S) Sulphate, British Journal of Dermatology, **81**, **Suppl. 4**, 63, 1969.

Fabin B., and Touitou, E., Localization of Lipophilic Molecules Penetrating Rat Skin In Vivo by Quantitative Autoradiography, International Journal of Pharmaceutics, **74**, 59, 1990.

Faratash, M., and Ponec, M., Improved Barrier Structure Formation in Air-Exposed Human Keratinocyte Culture Systems, Journal of Investigative Dermatology, **102**, 366, 1994.

Feldmann, R. J., and Maibach, H. I., Absorption of Some Organic Compounds Through the Skin in Man, Journal of Investigative Dermatology, **54**, 399, 1970.

Feldmann, R. J., and Maibach, H. I., Percutaneous Penetration of ^{14}C-Hydrocortisone in Man, Archives of Dermatology, **94**, 649, 1966.

Fleischmajer, R., Contard, P., Schwartz,E., Douglas MacDonald II, E., Jacobs II, L., and Sakai, L., Elastin-Associated Microfibrils (10 nm) in a Three-Dimensional Fibroblast

Culture, Journal of Investigative Dermatology, **97**, 639, 1991.

Flynn, G. L. and Yalkowsky, S. H., Correlation and Prediction of Mass Transport Across Membranes I: Influence of Alkyl Chain Length on Flux-Determining Properties of Barrier and Diffusant, Journal of Pharmaceutical Sciences, Vol 61, **6**, 838, 1972.

Flynn, G. L., General Introduction and Conceptual Differentiation of Topical and Transdermal Drug Delivery Systems: Differentiation With Respect to Delivery Kinetics, in **Topical Drug Bioavailability, Bioequivalence and Penetration**, Eds., V. P. Shah and H. I. Maibach, Plenum Press, New York and London, 1993, Chapter 20, pg. 370.

Francoeur, M. L., and Potts, R. O., Transdermal Flux-enhancing Pharmaceutical Compositions, U. S. Patent 86-925641, 1986.

Friedlander, S., Dhupar, K. C., Kumar, S., Malick, A. W., and Behl, C. R., Skin Uptake Studies: In Vitro Permeation and Retention of Acid and Salt Forms of Ro 23-3544 from Formulations Using Hairless Guinea Pig Skin and Finite Dose Methodology. Presented at the Eastern Region American Association of Pharmaceutical Scientists, NJ, 1988.

Garret E. R. and Chemburkar, P. B., Evaluation, Control and Prediction of Drug Diffusion Through Polymeric Membranes II. Diffusion of Aminophenone Through Silastic Membranes-A Test of the pH Partition Hypothesis, Journal of Pharmaceutical Sciences, **57**, 949, 1968.

Ghanem, A. H., Mahmoud, H., Higuchi, W. I., Rohr, U. D., Borsadia, S., Liu, P., Fox, J., and Good, W., The Effects of Ethanol on Transport of B-Estradiol and Other Permeants in Hairless Mouse Skin II. A New Quantitative Approach, Journal of Controlled Release, **6**, 75, 1987.

Golden, G., McKie, J., and Potts, R. O., Role of Stratum Corneum Lipid Fluidity in Transdermal Drug Flux, Journal of Pharmaceutical Sciences, **76(1)**, 25, 1987.

Good, W., Powers, M., and Campbell, P., A new Transdermal Delivery System For Estradiol, Journal of Controlled Release, **2**, 89, 1985.

Inagi, T., Muramatsu, T., Nagai, H., and Terada, H., Influence of Vehicle Composition on the Penetration of Indomethacin through Guinea-Pig Skin, Chemical and Pharmaceutical Bulletin,

29 (6), 1708, 1981.

Jacob, S. W., and Herschler, R., Eds., Biological Actions of Dimethyl Sulfoxide, Annals of New York Academy of Sciences, 243, 1975.

Jacob, S. W., and Rosenbaum, E. E., Biological Actions of Dimethyl Sulfoxide, Annals of New York Academy of Sciences, **141**, 1, 1967.

Jacob, S. W., and Wood, D. C., Notes on DMSO Symposium, Current Therapeutic Research, **9**, 299, 1967.

Jacob, S. W., Rosenbaum, E. E., and Wood, D. C., Dimethyl Sulfoxide, Vol. 1., Basic Concepts, Dekker, New York, 1971.

Hatanaka, T., Inuma, M., Sugibayashi, K., and Morimoto, Y., Prediction of Skin Permeability of Drugs. I. Comparison with Artificial Membrane, Chemical and Pharamceutical Bulletin, **38**, 3452, 1990.

Hayashi, R., Sugibayashi, K., and Morimoto, K., Calculation of Skin Permeability Coefficient for Ionized and Unionized Species of Indomethacin, Chemical and Pharamceutical Bulletin, **40**, 3090, 1992.

Hofland, H. E. J., van der Geest, R., Bodde, H., Junginger, H. E., and Bouwstra, J. A., Estradiol Permeation From Nonionic Surfactant Vesicles Through Human Stratum Corneum, Pharmaceutical Research, **11**, 659, 1994.

Holbrook, K. A., and Wolff, K., The Structure and Development of Skin, in **Dermatology in General Medicine**, Eds., T. B. Fitzpatrick, A. Z. Eisen, K. Wolff, I. M. Freeburg, and K. F. Ausen, McGraw Hill, New York, 1987. page. 93.

Hori, J., Linewar-Burke Analysis of Skin Penetration Enhancement, Journal of Controlled Release, **16**, 263, 1991.

Hunziker, N., Feldmann, R., Maibach, H. I., Animal Models of Percutaneous Penetration: Comparision of Mexican Hairless Dogs and Man, Dermatologica, **156**, 79, 1978.

Johnson, R., Nusbaum, B. P., Horowitz, S. N. and Frost, P., Transfer of Topically Applied Tetracycline in Various Vehicles Archives of Dermatology, **119**, 660, 1983.

Kadir, R., Stempler, D., Liron, Z., and Cohen, S., Penetration of Adenosine into Excised Human Skin from Binary Vehicles: The

Enhancement Factor, Journal of Pharmaceutical Sciences, **76(1)**, **77 (5)**, 409, 1988.

Kaidbey, K.H., Kligman, A.M., Topical Photosensitizers: Influence of Vehicles on Penetration, Archives of Dermatology, **110**, 868, 1974.

Kasting, G. B., and Robinson, P. J., Can We Assign an Upper Limit to Skin Permeability?, Pharmaceutical Research, **10**, 57, 1993.

Kleinman, H. K., Klebe, R. J., and Martin, G. R., Role of Collegenous Matrices in the Adhesion and Growth of Cells, Journal of Cellular Biology, **88**, 473, 1981.

Kligman, A. M., Dimethyl Sulfoxide, Part 2., Journal of American Medical Association, **193**, 923, 1965.

Knutson, K., Potts, R. O., Guzek, D. B., Golden, G. M., Mckie, J. E., Lambert, W. J. and Higuchi, W. I., Macro and Molecular Physical-Chemical Considerations in Understanding Drug Transport in the Stratum Corneum, Journal of Controlled Release, **2**, 67, 1985.

Kobayashi, D., Matsuzawa, T., Sugibayashi, K., Morimoto, Y., and Kimura, M., Analysis of the Combined Effect of 1-Menthol and Ethanol as Skin Permeation Enhancers Based on a Two-Layer Skin Model, Pharmaceutical Research, **11**, 96, 1994.

Koo Lee, C., Uchida, T., Noguchi, E., Kim, N.S., and Goto, S., Skin Permeation Enhancement of Tegafur by Ethanol/Panasate 800 or Ethanol/Water Binary Vehicle and Combined Effect of Fatty Acids and Fatty Alcohols, Journal of Pharmaceutical Sciences, **82**, 1155, 1993.

Koyama, Y., Bando, H, Yamashita, F., Takakura, Y., Sezaki, H., and Hashida, M., Comparative Analysis of Percutaneous Absorption Enhancement by d-limonene and Oleic Acid Based on a Skin Diffusion Model, Pharmaceutical Research, **11**, 13, 1994.

Krill, S., Knutson, K., and Higuchi, W. I., Effects of Ethanol on the Stratum Corneum Lipid Domain, Pharmaceutical Research, **5(10)**, S129, 1988.

Krowczynski, I. and Stozek, T., Liposomen als Wirkstofftrager in der percutanen Therapie, Pharmazie, **39**, 627, 1984.

Kubota, K., and Maibach, H. I., Significance of Viable Skin Layers in Percutaneous Permeation and Its Implication in

Mathematical Models: Theoretical Consideration Based on Parameters for Betamethasone Valerate, Journal of Pharmaceutical Sciences, **83**, 1300, 1994.

Kumar, S., Behl, C. R., Patel, S. B., and Malick, A. W., A Simple Microwave Technique for the Separation of Epidermis and Dermis in Skin Uptake Studies, Pharmaceutical Research, **6**, 740, 1989.

Kumar, S., Char, H., Patel S. B., Piemontese, D., Malick, A. W., and Behl, C. R., In Vitro Transdermal Delivery of 14C-Clonazepam Across Hairless Guinea Pig Skin, Presented at the 6th Annual American Association of Pharmaceutical Scientists Meeting held in Washington DC, 1991. Abstract # PDD 7316.

Kumar, S., Char, H., Mehta, D., Patel, S., Ciccone, G., Piemontese, D., Malick, A. W., and Behl, C. R., Permeability of Test Compounds Across Hairless Guinea Pig Skin and Living Skin Equivalent (TestSkin™), Presented at the 6th Annual American Association of Pharmaceutical Scientists Meeting held in Washington, 1991.

Kumar, S., Malick, A. W., Meltzer, N. M., Mouskountakis, J. D., and Behl, C. R., In Vitro Skin Permeation and Retention of a Leukotriene Antagonist from Topical Vehicles using Hairless Guinea Pig Model, Journal of Pharmaceutical Sciences, **69**, 631-37, 1992.

Kumar S., Weinrib, A. B., Dhupar, K. C., Kastrunis, L. M., Mouskountakis, J. D., Behl, C. R., Malick, A. W., Franz, T. J., Evaluation of 14C Ro 10-1670 In Vitro Transport From Various Topical Vehicles Through Hairless Mouse Skin. Presented at the 2nd Annual meeting of the American Association of Pharmaceutical Scientists, Boston, Massachussetts, Pharmaceutics Drug Delivery Abstract PD-517, June 1987.

Kurihara-Bergstrom, T., Woodworth, M., Feisullin, S., and Beall, P., Characteristics of the Yucatan Miniature Pig Skin and Small Intestine for Pharmaceutical Applications, Laboratory Animal Sciences, **36**, 396, 1986.

Lampe, M. A., Williams, M. L., and Elias, P. M., Human Epidermal Lipids: Characterization and Modulation During Differentiation, Journal of Lipid Research, **24**, 131, 1983.

Landeen, L. K., Zeigler, f. C., Halberstadt, C., Cohen, and Slivka, S. R., Characterization of a Human Dermal Replacement, Wounds, **4**, 167, 1992.

Laufer, L., DeFazio, J., Lu, Meldrum, D., Eggene, P., Sambhi, M., Hershman, J., and Judd, H., Estrogen Replacement Therapy by Transdermal Estradiol Administration, American Journal of Obstretics and Gynecology, **146(5)**, 533, 1983.

Lehman, P. A., and Franz, T. J., Effect of Age and Diet on Stratum Corneum Barrier Function in the Fischer 344 Female Rat, Journal of Investigative Dermatology, **100**, 200, 1993.

Levinson, K. K., Takayama, K., Isowa, K., Okabe, K., and Nagai, T., Formulation Optimization of Indomethacin Gels Containing a Combination of Three Kinds of Cyclic Monoterpenes as Percutaneous Penetration Enhancers, Journal of Pharmaceutical Sciences, **83**, 1367, 1994.

MacKee, G. M., Sulzberger, M. B., Herrmann, F., and Baer, R. L., Histologic Studies on Percutaneous Penetration With Special Reference to the Effect of Vehicles, Journal of Investigative Dermatology, **6**, 43, 1945.

Maibach, H. I., Feldman, R. J., Milby, T. H., Serat, W. F., Regional Variations in Percutaneous Penetration in Man. Pesticides, Archives of Environmental Health, **23**, 208, 1971.

Marzulli, F. N., Brown, D. W. C. and Maibach, H. I., Techniques for Studying Skin Penetration, Toxicology and Applied Pharmacology, Suppl., **3**, 76-80, 1969.

Masini, V., Bonte, F., Meybeck, A., and Weipierre, J., Cutaneous Bioavailability in Hairless Rats of Tretinoin in Liposomes or Gel, Journal of Pharmaceutical Sciences, **82**, 17, 1993.

McKenzie, A. W. and Atkinson, R. M., Topical Activities of Betamethasone Esters in Man, Archives of Dermatology, **89**, 711, 1964.

Mehta, D. B., Kumar, S., Patel, S. B., Piemontese, D., Char, H., Meltzer, N. M, Malick, A. W., and Behl, C. R., In Vivo Retention of Ro 24-0238 in the Epidermis and Dermis From Various Topical Vehicles Using Female Hairless Guinea Pig Model. Submitted to Journal of Pharmaceutical Sciences for publication in 1994.

Mehta, D. B., Kumar, S., Patel, S. B., Piemontese, D., Char, H., Meltzer, N. M, Malick, A. W., and Behl, C. R., In Vivo Retention of a Lipoxygenase Inhibitor in the Epidermis and Dermis From Various Topical Vehicles Using Female Hairless Guinea Pig Model. Submitted to Journal of Pharmaceutical Sciences for publication in 1994.

Menczel, E., and Maibach, H. I., In Vitro Human Percutaneous Penetration of Benzyl Alcohol and Testosterone: Epidermal-Dermal Retention, Journal of Investigative Dermatology, **54 (5)**, 1970.

Meyer, W., Schwarz, R., and Neurand, K., The Skin of Domestic Mammals as a Model for Human Skin, With Special Reference to the Domestic Pig, in **Current Problems in Dermatology**, Ed., G. S. Simmons, Z. Paster, M. A. Klingberg, and M. Kaye, **7**, Basel, Karger, 1978.

Mollgaard B., and Hoelgaard, A., Vehicle Effect on Topical Drug Delivery II. Concurrent Skin Transport of Drugs and Vehicle Components, Acta Pharmaceutics Seuca, **20**, 443-50, 1983.

Mollgaard, B. and Hoelgaard. A., Permeation of Estradiol Through the Skin-Effect of Vehicles, International Journal of Pharmaceutics, **15**, 185, 1983.

Monteiro-Riviere, N. A., Inman, A. O., Riviere, J. E., McNeill, S. C., and Francouer, M. L., Topical Penetration of Piroxicam Is Dependent on the Distribution of the Local Cutaneous Vasculature, Pharmaceutical Research, **10**, 1326, 1993.

Morimoto, Y. , Hatanaka, T., Sugibayashi, K., and Omiya, H., Prediction of Skin Permeability of Drugs: Comparison of Human and Hairless Rat Skin, Journal of Pharmacy and Pharmacology, **44**, 534, 1992.

Nacht, S., and Katz, M., in "Topical Drug Delivery Formulations", Ed., Osborne, D.W., Marcel Dekker, Chapter 15, 299, 1992.

Niazi, M. L., Molokhia, A. M., and El-Gorashi, A. S., Effect of Azone^{R} and Other Penetration Enhancers on the Percutaneous Absorption Dihydroergotamine, International Journal of Pharmaceutics, **56**, 181, 1989.

Nicolau, G., Dahlin, D.C., Kohlbrenner, M., Chan, P. S., Ronsberg, M. A., Saunders, T. K., Yacobi, A., and Cervoni, P., Skin Metabolism and Transdermal Absorption of Viprostol, a Synthetic PGE2 Analog, in the Rat: Effect of Vehicle, Skin Pharmacology, **2(1)**, 22, 1989.

Oberle, R. L., Das, H., Wong, S. L., Chan, K. K. H., and Sawchuk, R. J., Pharmacokinetics and Metabolism of Diclofenac Sodkum in Yucatan Miniature Pigs, Pharmaceutical Research, **11**, 698, 1994.

Okamoto, H., Muta, K., Hashida, M., and Sezaki, H.,

Percutaneous Penetration of Acyclovir Through Excised Hairless Mouse and Rat Skin: Effect of Vehicle and Percutaneous Penetration Enhancer, Pharmaceutical Research, 64, 1990.

Organogenesis, Inc., 83, Rogers St., Cambridge, MA 02142.

Ostrenga, J., Haleblian, J., Poulsen, B. J., Ferrell, B., Mueller N., and Sastri S., Vehicle Design for a New Topical Steroid, Fluocinonide, Journal of Investigative Dermatology, **56 (5)**, 392, 1971.

Patel, R. A., and Vasavada, R. C., Transdermal Delivery of Isoproterenol Hydrochloride: An Investigation of Stability, Solubility, Partition Coefficients and Vehicle Effects, Pharmaceutical Research, **5**, 116, 1988.

Pershing, L. K., In Vitro-In Vivo Correlation of Percutaneous Absorption with Human Skin. AAPS/FDA/AAD/SPS/USEHA Workshop, May 1989, Washington, DC.

Pershing. L., Lambert, L., and Knutson, K., Mechanism of Ethanol-Enhanced Estradiol Permeation Across Human Skin In Vitro, Pharmaceutical Research, **7(2)**, 170, 1990.

Physicians Desk Reference, 1994.

Portnoy, B., The Effect of Formulation on the Clinical Response to Topical Fluocinolone Acetonide, British Journal of Dermatology, **77**, 579, 1965.

Poulsen, B. J., Young, E., Coquilla. V., and Katz, M., Effect of Topical Vehicle Composition on the In Vitro Release of Fluocinolone Acetonide and its Acetate Ester, Journal of Pharmaceutical Sciences, **57**, 928, 1968.

Reifenrath, W. G., Chellquist, E. M., Shipwash, E. A., Jederberg, W. W., and Kruger, G. G., Percutaneous Absorption in Hairless Dog, Weanling pig and Grafted Athymic Nude Mouse: Evaluation of Models for Predicting Skin Penetration in Man, British Journal of Dermatology, **3**, 27, 123, 1984.

Reifenrath, W. G., Lee, B., Wilson, D. R., and Spencer, T. S., A Comparison of In Vitro Skin-Penetration Cells, Journal of Pharmaceutical Sciences, **83**, 1229, 1994.

Reifenrath, W. G. and Hawkins, G. S., The Weanling Yorkshire Pig as an Animal Model for Measuring Percutaneous Penetration, in **Swine in Biomedical Research**, Ed., M. E. Tumbleston, 1, Plenum Press, New York, 673, 1980.

Reifenrath, W. G., Hawkins, G. S., and Kurtz, M. S.,

Percutaneous Penetration and Skin Retention of Topically Applied Compounds: An In Vitro-In Vivo Study, Journal of Pharmaceutical Sciences, **80**, 526-30, 1991.

Rischel, W. A., Nathan D., and Hussain, A., Comparison of Skin Permeability for Two Model Substances Using Excised Skin From 5 Mammalian Species, Abs. # 1001, Presented at the 3rd American Association of Pharmaceutical Scientists Meeting, Oct, 1988.

Rolland, A., Wagner, N., Chatelus, C., Shroot, B., Site-Specific Drug Delivery to Pilosebaceous Structures Using Polymeric Microspheres, Pharmaceutical Research, **10**, 1389, 1993.

Rougier, A., Dupuis, D., Lotte, C., and Roguet, R., The Measurement of the Stratum Corneum Reservoir. A Predictive Method for In Vivo Percutaneous Absorption Studies: Influence of Application Time, Journal of Investigative Dermatology, **84**, 66, 1985.

Rougier, A., Lotte, C., and Maibach, H. I., The Hairless Rat: A Relevant Animal Model to Predict In Vivo Percutaneous Absorption in Humans, Journal of Investigative Dermatology, **88**, 577, 1987.

Rougier, A., Dupuis, D., Lotte, C., Roguet R., and Schaefer, H., In Vivo Correlation Between Stratum Corneum Reservoir Function and Percutaneous Absorption, Journal of Investigative Dermatology, **81**, 275, 1983.

Sarkany, I. and Hadgraft, J. W., The Influence of Formulation on Topical Corticosteroid Activity, British Journal of Dermatology, **81**, **Suppl 4**, 98-102, 1969.

Scala, J., McOsker, D. E., Reller, H. H., The Percutaneous Absorption of Ionic Surfactants, Journal of Investigative Dermatology, **50(5)**, 371, 1968.

Schaefer, H., Zesch, A., and Stuttgen, G., in **Skin Permeability**. Vol II. Publisher: Springer-Verlag, New York, NY. pp 1-2. 1982.

Scheuplein, R. J. and Ross, L. W., Mechanisms of Percutaneous Absorption. V. Percutaneous Absorption of Solvent Deposited Solids, Journal of Investigative Dermatology, **62**, 353-360, 1974.

Scheuplein, R. J. and Ross, L. W., Effects of Surfactants and Solvents on Permeability of Epidermis, Journal of Society of Cosmetic Chemists, **21**, 853, 1970.

Scheuplein, R. J., and Blank, I. H., Mechanisms of Percutaneous Absorption. IV. Penetration of Nonelectrolytes (Alcohols) From Aqueous Solutions and Pure Liquids, Journal of Investigative Dermatology, **60(5)**, 286, 1973.

Schuckler, F., and Lee, G., Measuring the Uptake of Azone into Excised Human Stratum Corneum from Thin Polymer Films, Journal of Pharmacy and Pharmacology, **45**, 162, 1993.

Schumacher, G. E., Drug Intelligence, **1**, 188, 1967.Seki, T., Sugibayashi, K., and Morimoto, Y., Effects of Solvents on Permeation of Nicardapine HCl Through Hairless Rat Skin, Chemical and Pharmaceutical Bulletin, **35(7)**, 3054, 1987.

Segal, R., and Pisanty, S., Glycerrhizin Gel as a Vehicle for Idoxuridine I. Clinical Investigations, Journal of Clinical Pharmacology and Therapeutics, **12(3)**, 165, 1987.

Shah, V. P., Behl, C. R., Higuchi, W. I. and Schaefer, H., Principles and Criteria in the Development and Optimization of Topical Therapeutic Products, Pharmaceutical Research, **9**, 1107, 1992.

Sherertz, E. F., Sloan, K. B., and McTierman, R. G., Effect of Skin Pretreatment With Vehicle Alone or Drug in Vehicle on Flux of a Subsequently Applied Drug: Results of Hairless Mouse Skin and Diffusion Cell Studies, Journal of Investigative Dermatology, **89**, 249, 1987.

Shroot, B., Chatelus, A., Civier and Hensby, C. N., A Cellulose Ester Film as an Inert Vehicle for Skin Pharmacological Studies, British Journal of Dermatology, III suppl., **27**, 163-7, 1984.

Slivka, S. R., Landeen, L. K., Zeigler, F., Zimber, M., and Bartel, R., Characterization, Barrier Function, and Drug Metabolism of an In Vitro Skin Model, Journal of Investigative Dermatology, **100**, 40, 1993.

Soter, N. A., Mast Cells in Cutaneous Inflammatory Disorders, Journal of Investigative Dermatology, **80**, 22s. 1983.

Sugibayashi, K., Sakonue, C., and Morimoto, Y., Utility of Topical Formulations of Morphine Hydrochloride Containing Azone^{R} and N-Methyl-2-Pyrrolidone, Sel. Cancer Therapeutics, **5**, 119, 1989.

Sugibayashi, K., Morimoto, Y., and Nemoto, M., Effect of Several Penetration Enhancers on the Percutaneous Absorption of Indomethacin in Hairless Rats, Chemical and Pharmaceutical Bulletin, **36**, 1519, 1988.

Sweeney, T. M., Downer, A. M., and Matolsty, A. G., The Effect of Dimethylsulfoxide on the Epidermal Water Barrier, Journal of Investigative Dermatology, **46**, 300, 1966.

Takahashi, K., Tamagawa, S., Katagi, T., Rytting, J. H., Nishihata, T., and Mizuno, N., Percutaneous Permeation of Basic Compounds Through Shed Snake Skin as a Model Membrane, Journal of Pharmacy and Pharmacology, **45**, 882, 1993.

Tiemessen, H. L. G. M., Bodde, H. E., Mollee, H., Junginger, H. E., A Human Stratum Corneum-silicone Membrane Sandwich to Simulate Drug Transport Under Occlusion, International Journal of Pharmaceutics, **53**, 119, 1989.

Tissot, J., and Osmundsen, P. E., Influence of Vehicles on the Topical Activity of Fluorometholone, Acta Dermatologica Venorology, (Stockholm), **46**, 447, 1966.

Touitou, E., Transdermal Delivery of Anxiolytics: In Vitro Skin Permeation of Midazolam Maleate and Diazepam, International Journal of Pharmaceutics, **33**, 37, 1986.

Touitou, E., Sink Permeation Enhancement by n-Decyl Methyl Sulfoxide: Effect of Solvent Systems and Insights on Mechanisms of Action, International Journal of Pharmaceutics, **43**, 1, 1988.

Treager, R. T., Factors Affecting Skin Permeability, Molecular Movement, in **Theoretical and Experimental Biology**, **5**, London, Academic Press, 1966, page 12.

Triglia, D., Braa, S. S., Donnelly, T., Kidd, I., and Naughton, G., A Three Dimensional Human Dermal Model Substrate For In Vitro Toxicological Studies, in In Vitro Toxicology: Mechanisms and New Technologies, Ed., Goldberg, A. M., Mary Ann Liebert, New York, 1991, Chapter 8.

Turi, J. S., Danielson, D., and Woltersom, J. W., Effects of Polyoxypropylene-15 Stearyl Ether and Propylene Glycol on Percutaneous Penetration Rate of Diflorasone Acetate, Journal of Pharmaceutical Sciences, **68**, 275, 1979.

Tymes, N.W., The Determination of Corticoids and Related Steroid Analogs by High-Performance Liquid Chromatography, Journal of Chromatographic Sciences, **5**, 151, 1977.

Vaidyanathan, R., Rajadhyaksha, V. J., Kim, B. K., Anisko, J. J., in **Transdermal Delivery of Drugs**, Eds., A. F. Kaydonius and B. Berner, CRC Press, Boca Raton, FL, 1987, Chapter 5.

Vasavada, R. C. and Patel, R. A., Investigation of Physicochemical and Formulation Parameters for Transdermal

Delivery of Isoproterenol Hydrochloride, Proceedings of Third European Congress of Biochemistry and Pharmacokinetics, **Vol. 1**, 1987, pp. 218.

Wahlgren, S., Lindstrom, A.L., and Friberg, S. E., Liquid Crystals as a Potential Ointment Vehicle, Journal of Pharmaceutical Sciences, **73(10)**, 1484, 1984.

Walters, M., Dugard, P. H., and Scott, R. C., In Vitro Percutaneous Absorption Studies: A Comparison of Human and Laboratory Species, Human Toxicology, **2**, 561, 1983.

Washitake, M., Anmo, T., Tanaka, I., Arita, T., and Nakano, M., Percutaneous Absorption of Drugs IV: Percutaneous Absorption of Drugs from Oily Vehicles, Journal of Pharmaceutical Sciences, **64**, 397, 1975.

Wearly L., and Chien, Y. W., Enhancement of the In Vitro Skin Permeability of Azidothymidine (AZT) via Iontophoresis and Chemical Enhancer, Pharmaceutical Research, **7 (1)**, 34, 1990.

Weinrib, A. B., Dhupar, K. C., Kastrunis, L. M., Kumar, S., Mouskountakis, J. D., Behl, C. R., Malick, A. W. and Franz, T. J., Evaluation of 14C-Ro 15-1570 In Vitro Transport From Various Vehicles Through Hairless Mouse Skin. Presented at the 2nd Annual meeting of the American Association of Pharmaceutical Scientists, Boston, Massachussetts, June 1987. Pharmaceutics Drug Delivery Abstract PD-516.

Wertz, P. W., and Downing, D. T., Glycolipids in Mammalian Epidermis: Localization and Relationship to Cornification, Science, **217**, 1261, 1982.

Wertz, P. W., Downing, D. T., Freinkel, R. K., and Traczyk, T. N., Sphingolipids of the Stratum Corneum and Lamellar Granules of Fetal Rat Epidermis, Journal of Investigative Dermatology, **83**, 193, 1985.

Wester, R. C., and Maibach, H. I., Percutaneous Absorption, in **Topical Drug Bioavailability, Bioequivalence and Penetration**, Eds., V. P. Shah and H. I. Maibach, Plenum Press, New York and London, 1993, Chapter 1, pg. 3.

Wester, R. C., Noonan, P. K., and Maibach, H. I., Variation in Percutaneous Absorption of Testosterone in the Rhesus Monkey Due to Anatomical Site of Application, Archives of Dermatological Research, **267**, 229, 1980.

Whitefield, M. and McKenzie, A. W., A New Formulation of 0.1 % Hydrocortisone Cream With Vasoconstrictor Activity and Clinical Effectiveness, British Journal of Dermatology, **92**, 585, 1975.

Wiechers, J. W., Absorption, Distribution, Metabolism, and Excretion of the Cutaneous Penetration Enhancer Azone, Ph. D. Thesis, University Centre for Pharmacy, Groningen, The Netherlands, 1989.

Wildnauer, R. H., and Kennedy R., Transepidermal Water Loss of Human New Borns, Journal of Investigative Dermatology, **54**, 483, 1970.

Wood, D. C. F., and Bettley, F. R., Effects of Various Detergents on Human Epidermis, British Journal of Dermatology, **84**, 320, 1971.

Woodford, R. and Barry, B. W., The Placebo Response to White Soft Paraffin/Propylene Glycol in the Skin Blanching Test, British Journal of Dermatology, **89**, 53, 1973.

Woodford, R. and Barry, B. W., Comparative Bioavailability of Proprietary Hydrophilic Topical Steroid Preparations, Journal of Pharmacy and Pharmacology, **Suppl 25**, 123, 1973.

Workshop on the Principles and Criteria in the Development and Optimization of Dermatological Products-Part I. Organized by the Food and Drug Administration (FDA and the American Association of Pharmaceutical Scientist (AAPS), Crystal City, Virginia, March 26-28, 1990.

Workshop on the Principles and Criteria in the Development and Optimization of Dermatological Products-Part II. Establishment of Bioavailability and Bioequivalence. Organized by the Food and Drug Administration (FDA and the American Association of Pharmaceutical Scientist (AAPS), Crystal City, Virginia, December 3-5, 1991.

Wuster, D. E and Kramer, S. F., Some Factors Affecting Percutaneous Absorption, Journal of Pharmaceutical Sciences, **50**, 28, 1961.

Yalkowsky, S. H. and Flynn, G. L., Transport of Alkyl Homologs Across Synthetic and Biological Membranes: A New Model for Chain Length-Activity Relationships, Journal of Pharmaceutical Sciences, **62 (2)**, 210, 1973.

Yamamoto, S., Jiang, H., and Kato, R., Stimulation of Hair Growth by Topical Application of FK506, a Potent Immunosuppresive Agent, Journal of Investigative Dermatology, **102**, 160, 1994.

Yamashita, F., Bando, H., Koyama, Y., Kitagawa, S., Takakura, Y., and Hashida, M., In Vivo and In Vitro Analysis of Skin Penetration Enhancement Based on a Two-Layer Diffusion Model

With Polar and Nonpolar Routes in the Stratum Corneum, Pharmaceutical Research, **11**, 185, 1994.

Yano, T., Higo, N., Fukuda, K., Tsuji, M., Noda, K., and Otagiri, M., Further Evaluation of a New Penetration Enhancer, HPE-101, Journal of Pharmacy and Pharmacology, **45**, 775, 1993.

Yu, D., Sanders, L. M., Davidson III, G. W. R., Marvin, M. J., and Ling, T., Percutaneous Absorption of Nicardapine and Ketorolac in Rhesus Monkeys, Pharmaceutical Research, **5 (7)**, 457, 1988.

Yum, S., Lee, E., Taskovich, L., and Theeuwes, F., Permeation Enhancement With Ethanol: Mechanism of Action Through Skin, in **Drug Permeation Enhancement**, Ed: D. S. Hsieh, Marcel Dekker, New York, Basle and Hong Kong, 1994, Chapter 8.

Ziegenmeyer, J., The Influence of the Vehicle on the Absorption and Permeation of Drugs, in **Dermal and Transdermal Absorption** (Ist International Symposium From 12-14 January, 1981, Munich), Eds: R. Brandau and B. Lippold, WVG mbH Stuttgart, 1982, Chapter 4.