

Introduction

Objective

Characterize the in vitro percutaneous absorption of human dermatomed skin, human dermis, ovine (sheep) vaginal, porcine (pig) buccal and newborn bovine (cow) corneal-scleral tissues following topical application of two model compounds. These tissues were selected for their utility as surrogate *in vitro* models in topical pharmaceutical product development for human use. Clotrimazole and hydrocortisone were selected as model compounds because of clinical use as topical anti-fungal and steroidal anti-inflammatory agents<sup>1</sup>, difference in partition coefficient, Log Ko/w<sup>2</sup> 6.26 (lipophilic) and 1.62 (hydrophilic), but comparable molecular weight<sup>3</sup> (344.8 and 362.5g/mol), respectively.

Methods

Tissue Source and Preparation

All tissues were obtained fresh. Human skin was obtained and prepared within 24 hours following elective surgery and stored frozen. Buccal, vaginal, corneal and scleral tissues were obtained fresh from local abattoirs. Animal tissues were prepared and stored under refrigeration a maximum of 24 hours prior to use. To maintain freshness the buccal, vaginal and human tissues were stored in vacuum sealed packages. Human skin and buccal tissue were dermatomed to a 0.813mm thickness (0.032"). The vaginal tissue was cleaned from surrounding connective tissue and muscle. The newborn bovine eyes were cleaned from exterior adipose and muscle tissue and stored whole under refrigeration, in a moist chamber. The whole globes were inspected for corneal epithelium damage using sodium fluorescein<sup>4</sup> (10% w/v in PBS) and examined under UV light. The scleral and corneal tissues were isolated and used the day of the study. Tissue thickness was measured randomly by a snap gauge micrometer.

Tissue Thickness (mm), Mean +/- SD	
Dermatomed Skin	0.803 +/- 0.091
Scleral	0.824 +/- 0.119
Buccal	0.919 +/- 0.178
Corneal	1.03 +/- 0.033
Vaginal	1.11 +/- 0.229
Dermis	1.96 +/- 0.563

Experimental Details

Clotrimazole and hydrocortisone emulsion creams (1% API<sup>1</sup>, 2% Isopropyl Myristate, 10% Stearyl alcohol, 5% Glycerin, q.s. deionized water) were manufactured with 1μCi/dose of <sup>3</sup>H Clotrimazole or <sup>3</sup>H Hydrocortisone. <sup>3</sup>H Clotrimazole or <sup>3</sup>H Hydrocortisone were added to the oil phase along with sufficient unlabeled API to achieve 1% API. After manufacture, creams were evaluated using a phase contrast microscope for physical homogeneity and then analyzed for radiolabeled API homogeneity.

All tissues were mounted in Bronaugh Flow-Through Diffusion Cells at 37°C and dosed with 5mg/cm<sup>2</sup> of either 1% 3H-clotrimazole or 1% 3H-hydrocortisone emulsion cream. Degassed receptor phase (Phosphate Buffered Saline with 0.1% Sodium Azide and 1.5% Oleth-20, pH 7.4) was pumped across the underside of the tissue at a rate of 1 - 1.5 mL/h.

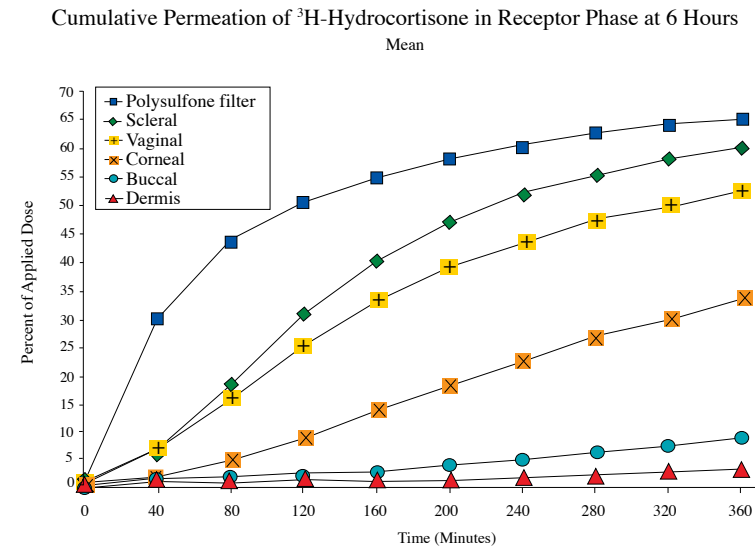
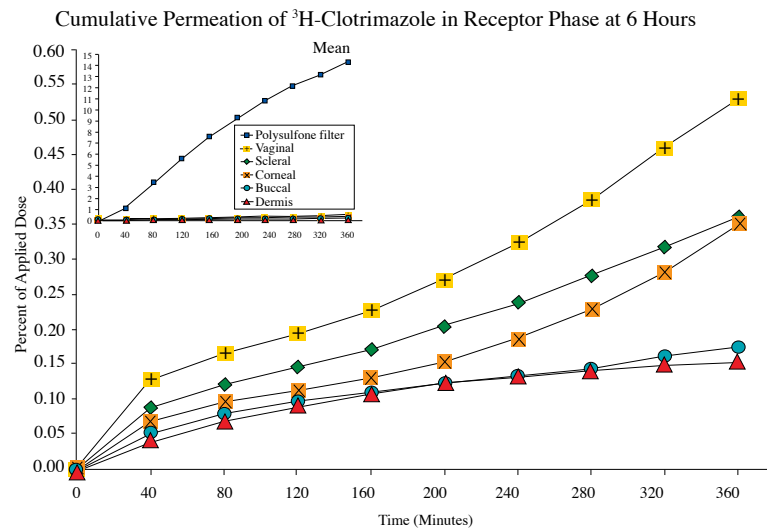
Duration of formulation exposure to each tissue was based on typical clinical use. Buccal, vaginal, dermis, corneal, scleral tissues and polysulfone filters (0.45μm pore size) were evaluated over a 6 hour exposure. Dermatomed skin, dermis, vaginal and buccal tissues were evaluated over the 24 hour exposure.



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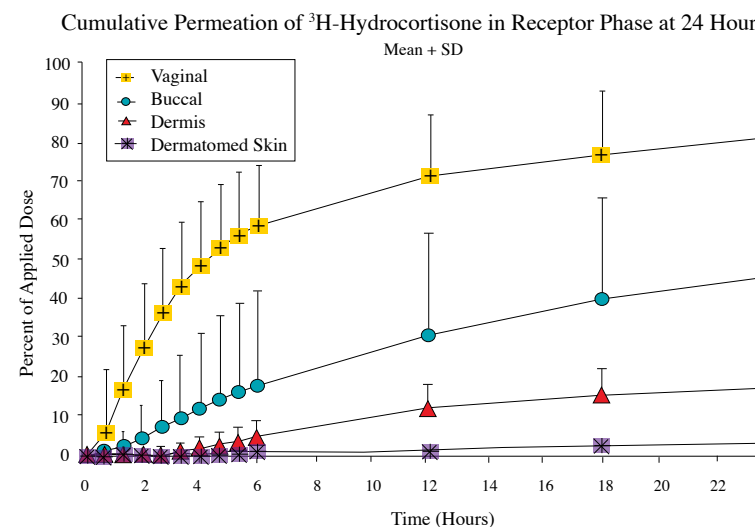
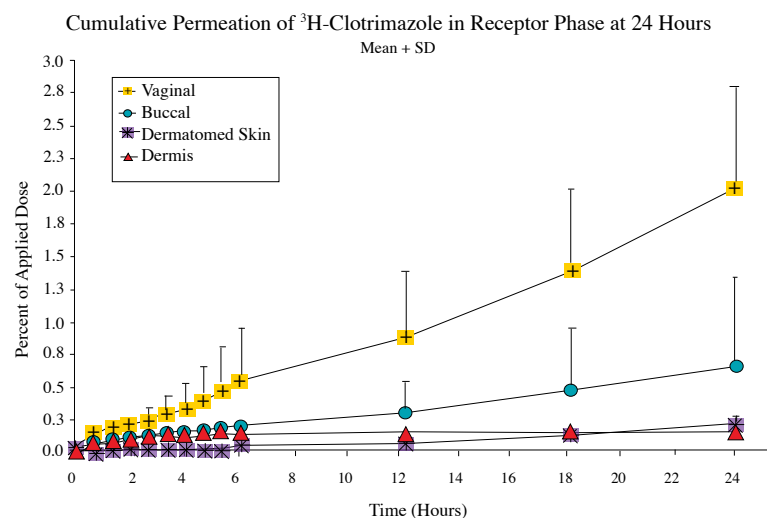
Results



	<sup>3</sup> H Clotrimazole Cumulative Applied Dose, Mean +/- SD					
	6 Hour Exposure			24 Hour Exposure		
	Receptor Phase	Tissue	Percent Recovery	Receptor Phase	Tissue	Percent Recovery
Polysulfone Filter	14.3 +/- 4.29	2.87 +/- 1.51	70.3 +/- 19.5			
Vaginal	0.530 +/- 0.407	5.13 +/- 3.05	98.9 +/- 11.5	1.74 +/- 0.495	6.73 +/- 3.56	102 +/- 8.47
Scleral	0.360 +/- 0.188	19.41 +/- 6.92	98.9 +/- 11.7			
Corneal	0.352 +/- 0.211	23.0 +/- 14.4	85.3 +/- 22.4			
Buccal	0.173 +/- 0.028	7.68 +/- 2.61	97.2 +/- 10.4	0.340 +/- 0.182	15.8 +/- 5.77	97.9 +/- 11.3
Dermis*	0.153 +/- 0.033	54.0 +/- 13.0	95.0 +/- 18.4	0.163 +/- 0.0295	55.4 +/- 25.6	95.6 +/- 6.76
Dermatomed Skin				0.197 +/- 0.0740		
					Epidermis	
					Dermis	
						95.6 +/- 6.23
					2.66 +/- 0.615	0.409 +/- 0.168

	<sup>3</sup> H Hydrocortisone Cumulative Applied Dose, Mean +/- SD					
	6 Hour Exposure			24 Hour Exposure		
	Receptor Phase	Tissue	Percent Recovery	Receptor Phase	Tissue	Percent Recovery
Polysulfone Filter	64.4 +/- 10.4	7.12 +/- 8.85	81.9 +/- 6.84			
Scleral	59.0 +/- 9.61	10.7 +/- 4.16	95.7 +/- 12.6			
Vaginal	51.5 +/- 13.6	11.0 +/- 10.5	89.2 +/- 7.36	62.2 +/- 7.13	3.00 +/- 1.20	87.8 +/- 7.19
Corneal	32.3 +/- 15.7	21.0 +/- 9.71	88.8 +/- 8.77			
Buccal	7.34 +/- 3.01	27.1 +/- 8.59	87.6 +/- 10.5	30.5 +/- 13.8	20.6 +/- 6.48	102 +/- 6.61
Dermis	1.94 +/- 1.23	72.3 +/- 10.71	91.3 +/- 7.68	10.3 +/- 4.37	63.5 +/- 5.88	89.1 +/- 13.8
Dermatomed Skin				2.44 +/- 1.48		
					Epidermis	
					Dermis	
						93.3 +/- 5.69
					3.09 +/- 0.916	0.842 +/- 0.732

\*Clotrimazole dermal permeation was rapid and complete after 4 hours.



Discussion

Vastly different levels of drug deposition into and permeation through the different tissues were observed; indicating the great importance of using the appropriate tissue model in an *in vitro* absorption study. In addition, results differed significantly across the two model compounds.

API octanol-water partition coefficient can affect permeation level as seen with these two compounds. Formulation composition and molecular weight can also affect permeation.

In most cases the rank order of permeation level across the different tissues for each compound was about the same. This indicates that the barrier function of the various tissues to the two different drugs in this formulation is similar. The observed differences in rank order suggest that the differences in tissue compositions play a significant role in barrier properties with respect to API's of different solubilities.

There is no correlation between thickness (physical pathlength of diffusion) of the various tissues and permeation level of API.

<sup>3</sup>H-Clotrimazole Permeation

In general, Clotrimazole exhibited a burst in tissue permeation within the first 40 minutes of exposure to the tissues. This burst in permeation was not observed with the highly permeable polysulfone filter membrane suggesting formulation component interaction with the biological tissues facilitating Clotrimazole permeation.

Clotrimazole permeation was low in the dermis-only tissue and virtually complete after 4 hours exposure. Cumulative permeation of Clotrimazole following 24 hours exposure to intact skin and dermis-only skin were comparable suggesting that the stratum corneum may not have a significant barrier function role to the prevention of the ingress of low molecular weight lipophilic drugs.

<sup>3</sup>H-Hydrocortisone Permeation

Across all tissue types, it is observed that Hydrocortisone penetrated well and reached steady-state permeation rate within the first six hours.

Tissue Deposition

The dermis-only deposition levels for both compounds were very high, but the dermis levels in the dermatomed skin were the lowest of all tissues. This shows that the epidermis serves as an effective barrier to dermal drug deposition.

The vaginal, buccal and dermis-only tissues were evaluated at both 6 hour and 24 hour exposure periods. By comparing the tissue data in the 6 and 24 hour studies we saw a few trends:

- Most of the <sup>3</sup>H-Clotrimazole tissue deposition seemed to occur during the first 6 hours with vaginal and dermis-only tissues. Only half of the buccal tissue's <sup>3</sup>H-Clotrimazole deposition occurred in the first 6 hours, the remaining occurred over the remaining 18 hours.
- There was more <sup>3</sup>H-Hydrocortisone vaginal, buccal and dermis-only tissue deposition in 6 hour study than the 24 hour study. This presents the notion that the <sup>3</sup>H-Hydrocortisone has penetrated into the tissue, but not permeated through the tissue into the receptor phase in the first 6 hours. The receptor levels at each collected time point validate that there was continual permeation through the entire 24 hour period.

Conclusions

Large permeation differences were detected across tissues and compounds. Clotrimazole showed a smaller range in permeation difference but relatively the same rank order across the various tissues relative to hydrocortisone. Permeation did not correlate with tissue thickness for either compound. There was no difference in Clotrimazole permeation between dermatomed skin and dermis; suggesting that highly lipophilic drug permeation is not limited by stratum corneum barrier function, but rather compound solubility and partitioning into the hydrophilic viable epidermis and dermis of the skin. The hydrophilic / lipophilic nature of these compounds did affect permeation and was dependent upon tissue composition as exemplified by comparison of vaginal with scleral tissues and dermis with dermatomed skin. The actual tissue barrier properties that affect drug deposition and permeation, especially in buccal and vaginal tissues, are not well characterized and warrant further investigation.

References

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