

# Potential Percutaneous Absorption Differences between Post Surgery Human Skin (Dermatomed, Dermis only and Heat Separated Epidermis), Rat Skin and Dermatomed Cadaver Skin, *In Vitro*, using <sup>3</sup>H-Clotrimazole and <sup>3</sup>H-Hydrocortisone as Model Compounds.

Marshall B, Lowe L, Larcom M, Winkle G, Bucks D  
Dow Pharmaceutical Sciences, Inc., Petaluma, CA 94954

## Objective

Potential differences in *in vitro* percutaneous absorption and permeation characteristics of two model compounds when using abdominal skin preparations from rats and humans, cadaver or post surgery, were evaluated following topical application. These tissues were selected based on their utility as proxy *in vitro* skin permeation models. Clotrimazole and hydrocortisone, both suspended at 1% in a cream (emulsion) formulation, were selected as model compounds<sup>1</sup> because of their physicochemical properties and clinical topical use as antifungal and corticosteroid anti-inflammatory agents. Clotrimazole is substantially more lipophilic than hydrocortisone with differences in partition coefficient (Log  $K_{ow}$ , 6.26 and 1.62), but similar molecular weights<sup>2</sup> (344.8 and 362.5 g/mol), respectively.

## Methods

### Tissue Source and Preparation

Only abdominal skin was utilized in this study. Skin from two human donors was obtained following elective surgery and dermatomed within 24 hours following surgery. The epidermis and approximately the upper third of the dermis were collected by dermatoming the skin at a setting of 0.813mm thickness (0.032") on a Padgett model-B dermatome and designated as human post surgery skin, also known as dermatomed skin. The remaining dermal tissue was collected and designated as dermis only. Tissues were stored at -20°C in vacuum-sealed packages prior to use. A portion of human post surgery skin #2 was used to prepare the heat separated epidermis. It was prepared by immersing the designated tissue into a water bath at 60°C for 55 seconds. This was followed by gentle peeling of the epidermis from the dermis using blunt dissection and rounded forceps. Human cadaver skin was obtained frozen from AlloSource, part code 5175-3R.N, ID: 081221-215, expiration date 1/30/2013. Full thickness rat skin, collected immediately following euthanasia, was supplied by Simonsen Labs, Gilroy CA (age 10-12 weeks, male, Sprague Dawley). Tissue thickness was measured using a snip gauge micrometer.

Abdominal Tissue Thickness (mm), Mean +/- SD	
Human Post Surgery Skin #1	0.792 +/- 0.106
Human Post Surgery Skin #2	0.984 +/- 0.098
Human Post Surgery Skin #2 Heat Separated	0.165 <sup>4</sup>
Human Cadaver Skin	0.418 +/- 0.184
Human Post Surgery Skin #3 Dermis Only	0.950 +/- 0.128
Sprague Dawley Rat Skin	0.612 +/- 0.068

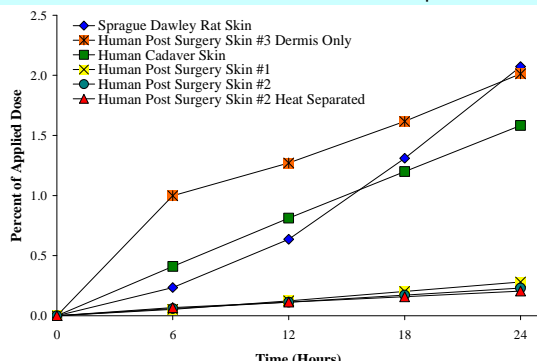
### Experimental Details

Clotrimazole and hydrocortisone emulsion creams (1% drug, 2% isopropyl myristate, 10% steryl alcohol, 15% emulsifying wax, 5% glycerin, 0.03% propylparaben, 0.17% methylparaben, q.s. purified 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 was added to the oil phase along with the same unlabeled drug. After compounding, radiolabel homogeneity in the creams was confirmed.

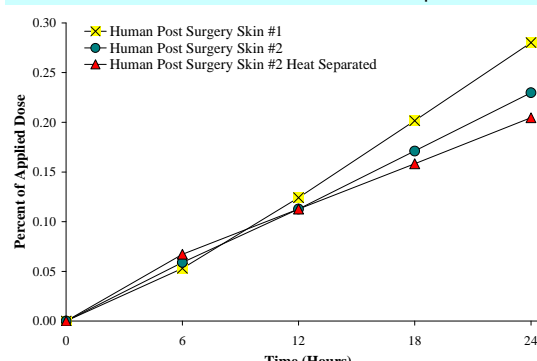
The heat separated epidermis was hydrated with receptor phase for one hour prior to dosing; a filter membrane was used to support this tissue in the diffusion cell. All tissues were mounted in Bronnugh flow-through diffusion cells (0.64 cm<sup>2</sup>) at 37°C and dosed with a clinically relevant amount, 5mg/cm<sup>2</sup>, of either 1% <sup>3</sup>H-clotrimazole or 1% <sup>3</sup>H-hydrocortisone emulsion cream. Fresh receptor phase (degassed phosphate buffered saline with 0.1% sodium azide and 1.5% oleth-20, pH 7.4) was pumped underneath the tissue at a flow rate of 1 mL/hr and collected in 6-hour intervals. After a 24-hour exposure period to the creams, residual formulation was removed from the skin surface and all samples were collected and analyzed for drug content.

## <sup>3</sup>H-Clotrimazole - 24 Hour Exposure

### Cumulative Permeation of <sup>3</sup>H-Clotrimazole in the Receptor Phase

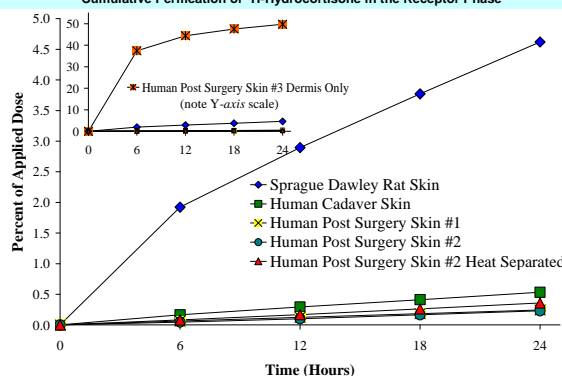


### Cumulative Permeation of <sup>3</sup>H-Clotrimazole in the Receptor Phase

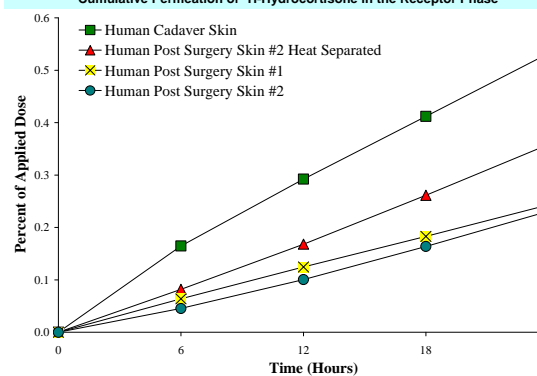


## <sup>3</sup>H-Hydrocortisone - 24 Hour Exposure

### Cumulative Permeation of <sup>3</sup>H-Hydrocortisone in the Receptor Phase



### Cumulative Permeation of <sup>3</sup>H-Hydrocortisone in the Receptor Phase



## References

1. Marketed product use level from the USP DI, Drug Information for the Health Care Professional, Volume I, Micromedex, 2007 27th Edition pp. 326, 862 and 936.
2. <http://www.appharmtech.com/view.asp?app=071014>
3. USP DI, Approved Drug Products and Legal Requirements, Volume III, Micromedex, 1999 19th Edition pp. IV138, and IV249
4. Bucks D. Prediction of percutaneous absorption. USM Dissertation Information Services. Ann Arbor, MI. (1989).
5. Magnusson BM, Anasjorn YG, Gross SE, and Roberts MS. Molecular size as the main determinant of solute maximum flux across the skin. J Invest Dermatol 2004; 122(4): 959-9.
6. Bucks D, Marshall B, Lane T, Winkle G. Stratum Corneum, Epidermis and Dermis Barrier Function: Implications in the Development of Topical Products Containing Large Molecular Weight Drugs The AAPS Journal Vol. 7, No. S1, Abstract W3053 (2005). Available from [www.aapsj.org/abstracts/NBCS\\_2005/NBCS\\_000602.pdf](http://www.aapsj.org/abstracts/NBCS_2005/NBCS_000602.pdf).

## Results

### <sup>3</sup>H Clotrimazole, Amount Absorbed as Percent of Applied Dose, Mean ± SD

Abdominal Tissue Type	Epidermis	Dermis	Receptor Phase
Human Post Surgery Skin #1	2.87 +/- 0.091	0.217 +/- 0.091	0.280 +/- 0.084
Human Post Surgery Skin #2	2.87 +/- 0.757	0.174 +/- 0.082	0.230 +/- 0.086
Human Post Surgery Skin #2 Heat Separated	1.21 +/- 0.412	0.205 +/- 0.084	
Human Cadaver Skin	11.4 +/- 6.92	0.631 +/- 0.312	1.58 +/- 0.735
Human Post Surgery Skin #3 Dermis Only		23.8 +/- 2.92	2.01 +/- 0.85
Sprague Dawley Rat Skin	42.4 +/- 10.5	3.51 +/- 2.09	2.07 +/- 0.657

### <sup>3</sup>H Hydrocortisone, Amount Absorbed as Percent of Applied Dose, Mean ± SD

Abdominal Tissue Type	Epidermis	Dermis	Receptor Phase
Human Post Surgery Skin #1	1.32 +/- 0.423	0.367 +/- 0.384	0.243 +/- 0.063
Human Post Surgery Skin #2	1.13 +/- 0.431	0.111 +/- 0.067	0.231 +/- 0.170
Human Post Surgery Skin #2 Heat Separated	1.71 +/- 1.14		0.358 +/- 0.259
Human Cadaver Skin	6.60 +/- 2.18	0.355 +/- 0.410	0.532 +/- 0.198
Human Post Surgery Skin #3 Dermis Only		8.19 +/- 2.71	49.8 +/- 11.5
Sprague Dawley Rat Skin	40.1 +/- 15.4	2.05 +/- 0.878	4.62 +/- 4.34

## Discussion

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

Cumulative permeation of clotrimazole across dermis only and rat skin were comparable. This level of permeation was substantially higher than in dermatomed skin. These observations suggested that the stratum corneum of the rat skin may not possess a significant barrier to prevent the entry of low molecular weight lipophilic compounds. Although numerically lower, permeation across dermis only skin was not statistically different compared to cadaver skin. This suggests that the stratum corneum of the cadaver skin may have been compromised during tissue procurement or post-mortem. Permeation differences of dermis only, cadaver and rat skin were statistically higher than both dermatomed skin donors and the heat separated epidermis. Permeation of both dermatomed skin donors and the heat separated epidermis were not statistically different and were essentially identical.

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

In contrast to clotrimazole, cumulative permeation of hydrocortisone across dermis only and rat skin were statistically different and were also both statistically different to all other tissue models tested. Permeation of both dermatomed skin donors were not statistically different and were essentially identical. Permeation of the heat separated epidermis was numerically greater than both dermatomed skin donors, but was not statistically different. The cadaver skin was statistically different to both dermatomed skin donors, but not to the heat separated epidermis. These observations suggest that there was very little donor-to-donor variability.

### Tissue Deposition

Tissue deposition of both compounds followed standard Fickian diffusion processes; as the permeation increased, the deposition of drug compound in the tissue increased. Removal of residual formulation from rat tissue was complicated by its higher follicular density and may have caused the apparently high epidermal levels.

## Conclusion

Dermis only, rat and cadaver skin were more permeable than dermatomed skin or heat separated epidermis. Clotrimazole exhibited less variation in permeation across the tissue types in relation to hydrocortisone. These observations indicated that the permeability of both compounds in each tissue type was similar despite the contrast in compound lipophilicity. This data is in agreement with Magnusson et al.<sup>5</sup> where molecular weight was defined as the primary determinant of maximum flux across human skin *in vitro*. The degree of permeation was related to tissue type and thickness rather than tissue thickness alone. Results obtained using dermatomed skin may be more indicative of clinical performance than cadaver or rat skin. The relatively high barrier function of dermatomed skin reduces the potential of falsely high permeation assessments of compounds undergoing topical formulation development. Conversely, cadaver and rat skin may be useful for toxicological evaluations because of the reduced likelihood of underestimating potential bioavailability. Heat separated epidermis is a feasible membrane for *in vitro* percutaneous absorption studies and is comparable to dermatomed tissue. Dermis only<sup>6</sup> tissue is an effective *in vitro* model for wounded skin.