

# Characterization of rabbit ear skin as a skin model for in vitro transdermal permeation experiments: histology, lipid composition and permeability

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## Introduction

The first step in the evaluation of a new transdermal formulation/molecule is that of in vitro permeation studies. The reference barrier for these studies is human skin, but its limited availability often necessitates the use of other skin models.

## Aim

The aim of this work was to characterize rabbit ear skin in order to verify if it is an acceptable model for human skin.

The characterization included:

- histological analysis
- determination of lipid composition
- permeation experiments

## Methods

### Lipid composition

Pre-weighed pieces of SC were extracted successively for 20 minutes in a sonication bath with chloroform:methanol 2:1, 1:1 and 1:2 v/v. HPTLC lipid separation was performed according to (1-3).

### Permeation experiments:

Franz type diffusion cells

Donor: 0.5 ml of nicotinamide water solution (200 mg/ml)

0.5 ml of caffeine saturated water solution ( $\approx$ 20 mg/ml)

0.5 ml of progesterone saturated solution in 0.4 HP $\beta$ CD water solution (229  $\mu$ g/ml)

Membrane: rabbit or pig ear skin, isolated pig epidermis

Receptor: saline solution (0.9% NaCl) for nicotinamide and caffeine; saline solution (0.9% NaCl) containing 0.4% of HP $\beta$ CD for progesterone

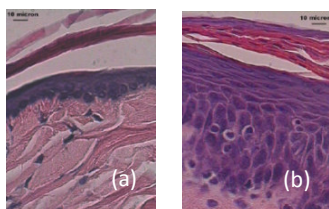
## Results

### Histology

Thickness ( $\mu$ m $\pm$ sd) of rabbit, pig, human and mouse skin.

|                  | Rabbit ear       | Pig ear           | Human | Mouse |
|------------------|------------------|-------------------|-------|-------|
| Stratum corneum  | 11.7 $\pm$ 1.7   | 9.1 $\pm$ 1.7     | 12.5  | 6.7   |
| Viable epidermis | 17.0 $\pm$ 2.6   | 61.7 $\pm$ 6.7    | 53.5  | 9.6   |
| Full thickness   | 276.4 $\pm$ 59.8 | 1177.1 $\pm$ 29.0 | nr    | nr    |

The structure and thickness of stratum corneum resulted comparable for pig and rabbit ear skin, whereas the other layers, namely viable epidermis and dermis, where different not only in terms of thickness but also as structure.



Sections of rabbit (a) and pig (b) ear skin (40x)

### Lipid composition

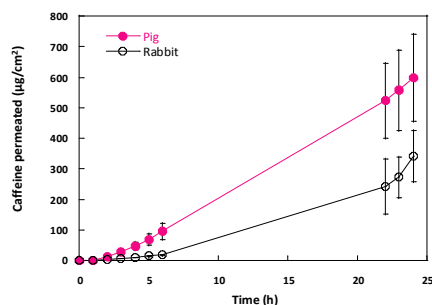
Stratum corneum lipid composition (% of total lipid content)

|   | Pig            | Rabbit          |
|---|----------------|-----------------|
| Ceramides NS                            | 20.7 $\pm$ 2.5 | 24.5 $\pm$ 0.9  |
| Ceramides AS                            | 22.7 $\pm$ 2.6 | 10.2 $\pm$ 0.9  |
| FFA (oleic acid)                        | 22.5 $\pm$ 1.1 | 16.3 $\pm$ 0.6  |
| Cholesterol                             | 31.5 $\pm$ 0.3 | 11.4 $\pm$ 0.8  |
| Triglycerides (trioleine)               | 1.1 $\pm$ 0.1  | 5.1 $\pm$ 2.4   |
| Cholesterol esters (Cholesterol oleate) | 1.5 $\pm$ 0.2  | 32.5 $\pm$ 11.4 |
| Squalene                                | 0              | 0               |

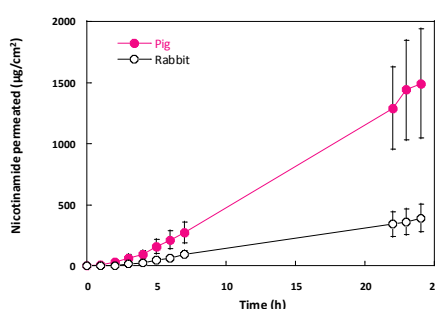
Despite the total amount of lipids extracted was similar in pig and rabbit SC (5 and 6% of the initial weight of SC for pig and rabbit respectively) notable differences exist in the composition of the two extracts. The overall results obtained indicate that rabbit SC is composed by a substantially higher percentage of nonpolar lipids, such as cholesterol esters and triglycerides, compared to pig skin.

## Permeability

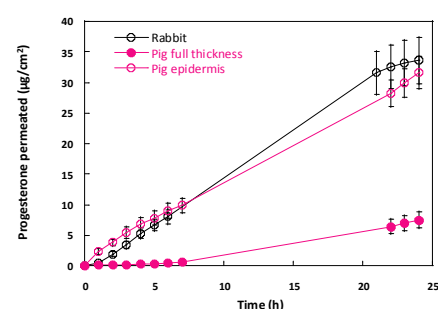
### Caffeine



### Nicotinamide



### Progesterone



## Conclusions

Overall the results obtained in this work support the usefulness of rabbit ear skin as barrier for skin penetration studies, for both lipophilic and hydrophilic permeants, even though its lipid composition is different from human skin.

## References

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- 3) Lampe MA, Burlingame AL, Whitney J, Williams ML, Brown BE, Roitman E, Elias PM, J Lipid Res 1983;24:120-130.