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SHORT REPORTS

Transfollicular Percutaneous Absorption

Skin Model for Quantitative Studies

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Theoretically percutaneous absorption comprises two components: the transepidermal route and the transappendageal route, so-called 'shunt' diffusion. The relative importance of 'shunt' diffusion was investigated with a model of appendage-free skin. After treatment with 60°C water for exactly 1 min, hairless dorsal rat skin regrows as a continuous epidermis. We present preliminary results of hydrocortisone diffusion in vitro, in appendage-free skin relative to normal skin. With intact skin under our experimental conditions, the steady-state flux and the total diffusion in 24 h were 50-fold larger. *Key words: Shunt diffusion; Appendage-free skin.* (Received January 29, 1988.)

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Percutaneous absorption theoretically comprises two components: the transepidermal route and the transappendageal route or 'shunt' diffusion (1). However, the relative importance of shunt diffusion has not yet been quantitatively investigated due to the lack of models allowing comparison of percutaneous absorption in skin with vs. without follicles. In the following we propose such a model and present our preliminary results.

Behl et al. (2) showed in hairless mice that, after removal by brief heat treatment, the epidermis reforms without follicles but with excessive contraction. This considerable contraction during regrowth leaves an area of follicle-free epidermis too small for meaningful experimentation. We therefore modified this technique, using hairless rats. Our technique reduces the contraction during regrowth in order to obtain a larger area of appendage-free skin adjacent to normal skin at the same site. We then used this skin to study the percutaneous absorption of hydrocortisone in vitro.

MATERIAL AND METHODS

Appendage-free skin on hairless rat

Female hairless rats (n=30, rat nu ICO; IFFA CREDO, F-69210 L'Arbresle; 6–8 weeks old) were anaesthetized (pentobarbital, 30 mg/kg) and treated by immersion of about 8 cm^2 in the middle of the back in 60°C water for exactly 1 min. The epidermis was then removed from this treated area and the dermis was protected with a bandage for one day to prevent scratching. Subsequent healing progressed without a bandage and was monitored for unnecessary animal distress.

The treated animals were kept in individual cages and examined every week. A thick scab covered the affected area after one week, dried gradually and finally sloughed off at about the third week post-treatment. The newly exposed surface appeared smooth, pinkish and contracted relative to the initial area by about one-third, corresponding to about 5 cm^2.

Rats (n=3) were sacrificed every week from the third week on and biopsies were then taken from both the intact and treated dorsal skin of each animal. The biopsies were examined histologically for integrity and the presence or absence of appendages. The thickness of the epidermis and dermis were measured.

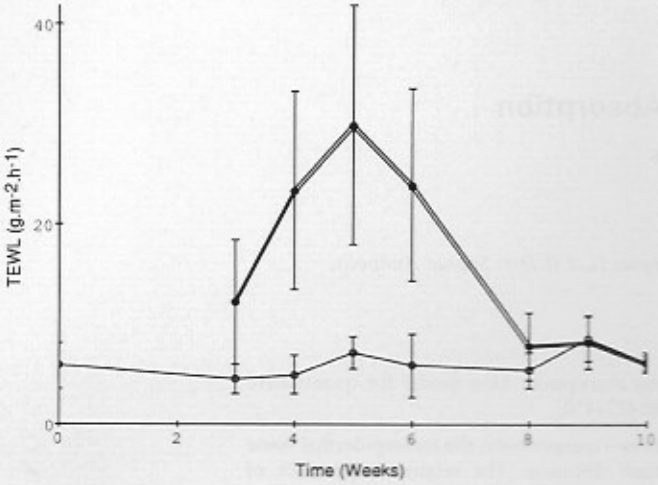

Fig. 1. Transepidermal water loss through intact (○—○) or regrown (●=●) skin (n=6).

The thickness of the stratum corneum was evaluated, using light microscopy, by counting the layers of keratinocytes after swelling of frozen sections with aqueous NaOH 0.25 N.

The hyperplastic state of the epidermis was evaluated using a method of cross-linked envelope-counting (3).

The restoration of barrier function of the skin was followed in vivo by measuring the transepidermal water loss (TEWL) every week during regrowth since the TEWL is a measure of the functional integrity of the horny layer (4, 5).

Diffusion of hydrocortisone

The permeation of tritium-labelled hydrocortisone was investigated in vitro in appendage-free skin vis-à-vis normal skin. Animals were sacrificed 9 weeks after the heat treatment. Full-thickness samples of regrown as well as adjacent normal dorsal skin were taken and mounted on Franz diffusion cells.

The skin surface was exposed to ambient conditions. The dermis was bathed by isotonic saline and the temperature maintained at 32°C. Hydrocortisone was dissolved at 1% in an hydroalcoholic lotion and 50 µl (25 µCi) was applied onto the 2 cm^2 surface with 1 min of massage. Samples of the saline–receptor fluid were taken at 1 h intervals, for 24 h. In addition, samples of skin were taken at the end of the pene-

Table I. *Measurements of rat skin thickness; values are mean ±1/2. (95% confidence interval) (n=20)*

Skin sample	Epidermis (µm)	Whole skin (mm)
Intact skin	27.7±12.7	1.09±0.22
Regrown skin	38.2±21.1	0.95±0.15

Table II. *Diffusion of hydrocortisone; values are mean ±1/2. (95% confidence interval) (n=10)*

Skin sample	Steady-state flux (pmol · cm^{-2} · h^{-1})	Total diffusion after 24 h (nmol · cm^{-2})
Intact skin	602.5±252.4	15.01±4.63
Regrown skin	12.1±5.2	0.35±0.14

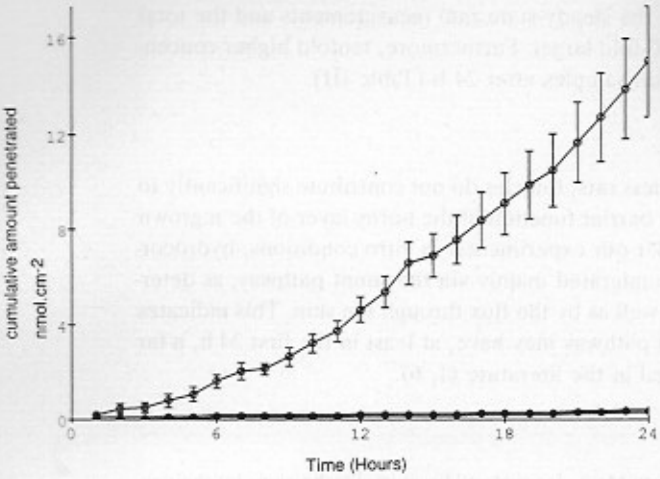

Fig. 2. Permeation profiles of hydrocortisone through the hairless dorsal intact (○—○) or regrown (●=●) skin at 32°C (n=10).

tration experiment, the horny layer was removed by stripping and the quantity of hydrocortisone in the epidermis and dermis was determined. All samples were analysed for their radioactivity content in a liquid scintillation counter.

RESULTS

Table I gives the values for the thickness of the epidermis and whole skin of intact and regrown dorsal skin, 9 weeks after treatment. The difference was not statistically significant.

Six weeks after treatment, we noticed a complete lack of appendages in the regrown skin. At this time, the stratum corneum was still hyperkeratotic; compared with normal skin, a thickened epidermis and a more compact and more vascularized dermis were noted. After 8–9 weeks, the epidermal–dermal junction became flat, the stratum corneum, epidermis and dermis had acquired normal thickness and appearance, but the dermis remained more vascularized and the size of intercellular spaces was normal. The increased vascularization of the regrown skin cannot interfere with absorption because vessels are not functional in this in vitro approach.

The hyperplastic state of the epidermis showed a normalization after 9 weeks of healing.

The level of water loss increased considerably just after the loss of the scab (third week after treatment) and returned to normal after 8 to 9 weeks (Fig. 1).

The data reported in Table II and in Fig. 2 show that the permeation of hydrocortisone through intact skin was considerably greater than that through appendage-free regrown skin.

Table III. *Hydrocortisone concentrations in the skin after 24 h; values are (μM) mean ±1/2. (95% confidence interval) (n=10)*

	Skin sample	
Tissue	Intact skin	Regrown skin
Epidermis[a]	320.60±111.11	9.84±2.33
Dermis	22.75±9.07	0.77±0.14
Whole skin	18.71±7.06	1.40±0.38

[a] The epidermal in vivo concentration under comparable experimental conditions was 204±109 μM (5).