

# CHANGES IN STRATUM CORNEUM AFTER UREA APPLICATION TO HUMAN SKIN IN VIVO. ELECTRON MICROSCOPIC INVESTIGATIONS

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## Synopsis

Urea, 10% in cream or ointment, applied in vivo for 24 and 48 hours with part occlusion changes the inner structure of the horny cells, depending on time and the excipients. Splitting of the keratin changes the matrix and the osmiophilic behaviour, especially in the upper regions. After 48 hours fine granulation within the horny cells and enhancement of cavities can be demonstrated. There is no evidence of any changes in the osmiophilic material in the intercellular spaces. Urea does not enhance permeability per se, it increases the surface of the keratinous material and its capacity to bind water and other substances with low molecular weight.

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## Riassunto

L'applicazione topica mediante bendaggio parzialmente oclusivo di una crema o di un unguento al 10% di urea provoca modificazioni della struttura dello strato corneo, dipendente dal tempo di contatto e dal tipo di veicolo. Si osserva del materiale cheratinico con variazioni a livello delle matrici. Dopo 48 ore si osserva una fine granulazione delle cellule cornee con comparsa di cavità. Non si evidenziano cambiamenti degli spazi intercellulari. L'urea non facilita di per se la permeabilità ma aumenta la superficie del materiale cheratinizzato e la sua capacità di legare acqua e altre sostanze a basso peso molecolare.

## Introduction

The influence of urea on the horny layer and the ensuing changes in function have been researched very carefully (1, 2, 3, 5). The external use of urea, incorporated into different bases, in dermatology and cosmetology depends on such data (6).

The changes in the structure of the horny layer after topical application of urea to human beings have not however, until now been examined electron microscopically, either in vivo or in vitro.

Stratum corneum can be divided into 3 layers (Orfanos 1981).

1. Flat horny cells in the basal zone having electron dense membranes which enclose the homogeneous relatively light material.

2. The middle zone consists of horny cells with an electron - dense network of different structures which can be interrupted by small cavities.

3. Finally the superficial zone is characterised by broad intercellular spaces and lack of desmosomes, which are visible as electron dense threads only in - the lower horny layer .

Tonofilaments and the hyaline granules are the material of which the keratin, in the form of bigger tonofibril hyalin complexes is composed. The formation of horny layer is a quick process, more or less a jump, into keratinisation.

Horny cells with an osmiophobic filaments, embedded in an osmiophilic matrix are known as A-cells. Horny cells with a pattern of osmiophilic filaments embedded in an osmiophilic matrix are classified as B-horny cells. The intercellular substance is formed by membrane coating granules (Odland-bodies) which are extruded into the intercellular space in the granular layer and form glycosphingolipids, ceramides, nonesterified sterols and fatty acids. This material forms the bilipid layers which as lamellar sheets surrounding the horny cells (4).

## Methods

We took punch biopsies (2mm in diameter) after application of 10% urea in creams or ointment for 24 hours or 48 hours and examined them by electron microscopy.

For the first 24 hours occlusive dressings were used, subsequently the applications were letopen. As a control the effect of cream or ointment without urea, was also examined.

Excised skin (for the region around a skin tumour) was used for an in vivo experiment at 30°C with the same conditions as in vitro.

The region used was to the right and left of the umbilical line and an area of 25 cm<sup>2</sup> was used for the applications.

The structure of the horny layer in the same area was examined prior to any applications. Basodexan cream and ointment with and without 10% urea were made available by the courtesy of Röhm Pharma. The experiment was performed on healthy probands, who gave their consent to the procedures.

The punch slices were cut with an ultratom DMU 3 (Reichardt) and examined by an electron microscope Dm 9 of Carl Zeiss. After fixation (2% glutaraldehyde, 2% O<sub>5</sub>SO<sub>4</sub> in phosphate buffer 7.4) the slices were embedded in araldite.

Parameters of the effect of urea are: the amount of electron dense material in the horny cells, including the cell envelopes, and of the intercellular substance, the thickness of the cells and the width of the intercellular space.

## Results

The composition of the horny layer structures of the skin treated with urea shows the following changes in comparison with the control skin:

The thickness of the horny cells has diminished, especially in the region towards the epidermis.

The number of horny layers, usually 11 to 12, is reduced to 8 to 10.

Additionally vacuolisation, development of cavities and changes to the inner structures towards the skin surface are obvious.

The density - the osmiophilic behaviour - changes and this is especially obvious in the horny layer, as is the smaller diameter of the transverse cross sections.

The intercellular substance and the intercellular

space show no changes. The width of the intercellular space seems in general not to be changed. The desmosomes, separated from the tonofibrils are now part of the intercellular material and disappear into the upper region of the horny layer (Fig. 1,2)

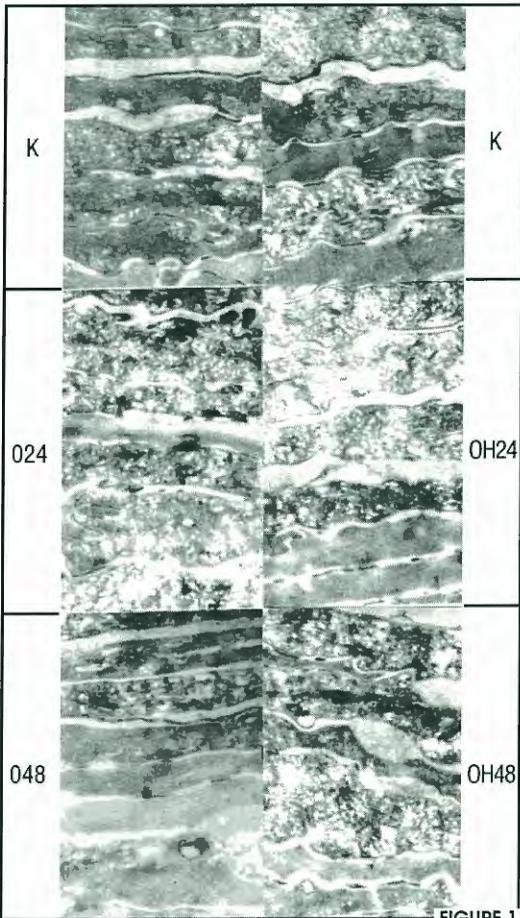


FIGURE 1

Urea ointment effect in vivo on human horny layers (x 23750)

K = Biopsy before treatment

O24 = 24 h after application of ointment without urea

OH24 = 24 h after application of 10% urea ointment

O48 = 48 h after application of ointment without urea

OH48 = 48 h after application of 10% urea ointment

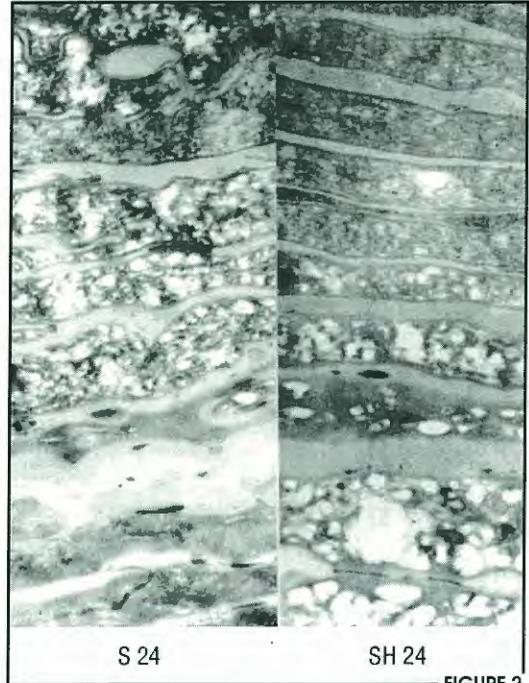


FIGURE 2

Urea ointment in vitro on human horny layer (x23750)

O24 = 24 h after application of ointment without urea

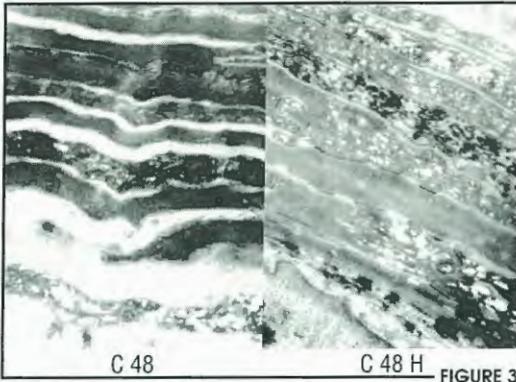
OH24 = 24 h after application of 10% urea ointment

O48 = 48 h after application of ointment without urea

OH48 = 48 h after application of 10% urea ointment

The swelling of the horny cells can be combined with bulging and narrowing of the intercellular space. It is obvious that the target of the urea is the horny cell, which show evidence of loosening of the hydrogen bonds of the keratin.

This phenomenon is obviously more expressed after 48 hours (occlusive dressings for 24 hours, followed by 24 hours open application). The envelopes remain intact, irrespective of cream, ointment or time. (Fig. 3)



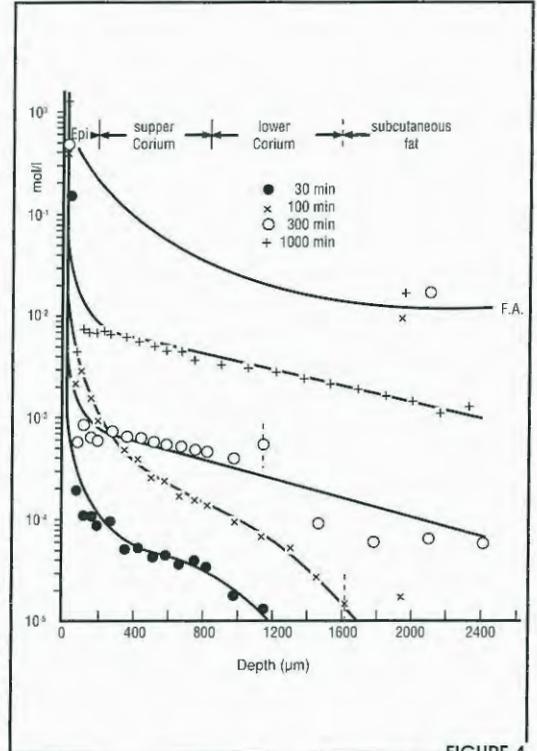
Urea cream effect in vivo on human horny layer (x 23750)  
 C48 = 48 h after application without urea  
 CH48 = 48 h after application of 10% urea cream

## Discussion

Urea applied in vivo and in vitro shows binding to filaments of the keratin and the envelopes around the keratin filaments which are less tightly packed, and permits accumulation of water in its variable forms. The inner structure of the horny cells has changed and shows clear "splitting" of the horny filaments. These effects of urea, replacing water on the one hand and binding water on the other, and the action as a solvent on the different micro elements of the cell provide the key to a broad spectrum of effects both medical and cosmetic importance (11, 12).

From the toxicological and histological points of view there are no objections in regard to an impaired barrier function using 10% urea which permeates the skin (Fig. 4). The intercellular spaces with their lipid layers and proteoglycans are adequate to inhibit uncontrolled permeation. Steroids show an increased rate using urea. On the other hand smaller molecules such as Imidazoles (Antimycotics) show an increased accumulation in the horny layer and a smaller flux towards the corium (10).

The effects of urea do not greatly differ between



Permeating of urea 10% cream  
 Examinations with chamber technique in vitro using human skin (abdomen)  
 Application of 5 mg cream/2 cm<sup>2</sup> = 250 μ urea/cm<sup>2</sup>  
 TA = stripped skin (8 x with Tesafilm)

living skin and excised skin in vitro. The water uptake from the subcorneal layers to the horny layer has also to be taken into consideration. The microbial material of the horny layer is also by urea.

The base in which urea is incorporated is responsible for the depth of its action which depends on the diffusion. Urea cannot be regarded as a general enhancer of permeability of the human stratum corneum in the same sense as dimethylsulfoxide (DMSO) or laurocapram (Azone). The intercellular space with its bilipid layers is only passively involved by swelling of the horny cells without any visible change of the envelope.

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