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Quantitative evaluation of urea in stratum corneum of human skin

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Urea, as a final product of protein metabolism, is a physiological substance in the human organism. The elimination of urea in humans amounts to 30 g per day; the amount in urine is of the order of 2% and in the blood 250 mg per ml. The urea content of human skin is about 1%.

Urea, as one of the natural moisturizing factors, influences the capacity of the horny layer to bind water and it also has keratoplastic, penetration-promoting, antimicrobial and antipruritic properties. Therefore, the observation that pathological skin alternations are accompanied by alterations in the content of urea is not unexpected. This has been studied and confirmed previously by several investigators using various analytical methods. Schwarz determined urea in aqueous extracts of scrapings from the horny layer of healthy volunteers and of patients with atopic dermatitis or psoriasis using thin layer chromatography followed by detection with Ehrlich's reagent [6] and, in a separate study, using an amino acid analyser [7]. For the investigation of urea content in aged skin Kügelgen and Schwarz [5] also used an amino acid analyser. The amount of urea in callus was determined by Jacobi [3] using urease [1].

The procedure applying the amino acid analyser permits a determination of urea values only related to the part of amino acids. A disadvantage of all the methods previously described is that, in using scrapings from the horny layer, only the very superficial parts of the stratum corneum are considered.

The analytical method described here allows the quantitative evaluation of urea in absolute values over the whole thickness of the horny layer in a defined area of 2.5 cm^2. The method was selected using the following the critera: detection limit, sensitivity, specifity, cost and time. The condensation of urea with diacetyl monoxime to a yellow-coloured compound measurable by its absorbance at a wavelength of 480 nm [2, 4] was tried but the detection limit was found to be the restricting factor. When carrying out the procedure in the presence of an aromatic amine [9] the maximum absorption of the resulting product is displaced to longer wave lengths with a large decrease in the detection limit. We used the aromatic amine *N*-(1-naphthyl)ethylenediamine dihydrochloride and, after optimization of the reaction conditions, achieved a detection limit of 0.5 µg urea per sample.

The horny layer samples were taken from the body site of interest using a template with a 2.5 cm^2 window. Histological examination of a skin specimen from the upper arm from which 20 strips had been taken demonstrated that the stratum corneum had been completely removed. The total number of horny layer strips from one skin site was divided consecutively into groups containing two, three, five and ten tesafilm pieces. This partitioning was chosen because of the decreasing adhesion of horny material to the film with increasing depth [11] and because of the detection limit of the analytical method for urea in the horny material.

The fractioned strips were extracted by shaking for 40 min with 3.0 ml distilled water and 4.0 ml chloroform in glass tubes. The urea was dissolved in the aqueous phase and the tesafilm components were concentrated in the chloroform. The samples were centrifuged at 3000 rpm for 5 min to separate the phases. For the quantification of the extracted urea, 1.0 ml diacetyl monoxime solution (100 mg per 100 ml water) was added to 2.0 ml of the aqueous phase together with 0.5 ml *N*-(1-naphthyl)-ethylenediamine dihydrochloride reagent (145 mg in 100 ml sodium hydrogen carbonate solution; 0.05% w/v) and 0.5 ml concentrated sulphuric acid. After mixing well, the glass tubes were stoppered and immersed in boiling water for 10 min. Aqueous potassium persulphate solution (0.25 ml, 1% w/v) was then added and, after 15 min under protection from the light, the resulting colour was measured spectrophotometrically at a wavelength of 564 nm. The method was calibrated with aqueous urea solutions of known concentration.

The recovery of the method was determined as fol-

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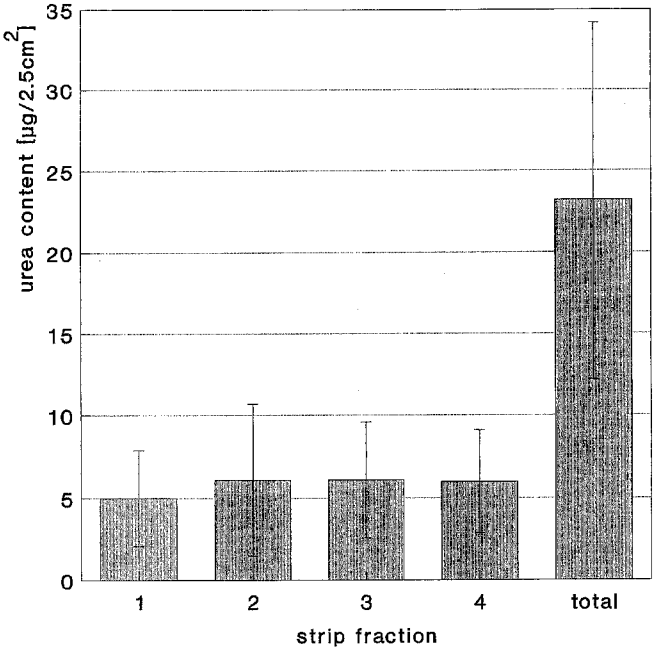

Fig. 1. Urea content of the horny layer of healthy volunteers ($n = 67$) *1*, strips 1 and 2; *2* strips 3–5; *3*, strips 5–10; *4*, strips 10–20

lows: 10 mg of an ointment containing 10% urea was applied to 4 cm^2 of excised mammalian skin, which was assumed to be urea-free before the application. After 1 hour for penetration the ointment was removed with a cotton swab and the unpenetrated urea remaining in the ointment quantified. The urea content of the skin was determined by the stripping technique as described above. The total of the two values from several independent tests showed the recovery to be complete. The absence of urea in the skin samples before application of the ointment was ensured by parallel analysis of untreated samples. Penetration of the applied urea into deeper skin layers was not expected because of the short penetration time [10].

The described method has been used success fully for various purposes, for example to evaluate the urea content in the stratum corneum of patients suffering from atopic dermatitis in comparison with healthy volunteers [8] as well as patients with psoriasis. The distribution of urea in fractionated strips taken from the upper arm of healthy volunteers is shown in Fig. 1. Studies of the effects of age and other aspects are in progress.

References

1. Fawcett J, Scott JE (1960) A rapid and precise method for the determination of urea. J Clin Pathol 13: 156–160
2. Horsch W, Wolf B (1985) Harnstoff. Eine Übersicht unter Berücksichtigung seiner pharmazeutischen Verwendung und Analytik. Pharmazie 40: 665–676
3. Jacobi O (1971) Die Inhaltsstoffe des normalen Stratum corneum und Callus menschlicher Haut. Arch Dermatol Res 240: 107–118
4. Kakác B, Vejdelek ZJ (1966) Handbuch der Kolorimetrie, Bd. III, pp 787–789
5. Kügelgen H, Schwarz E (1973) Zur Frage von Altersveränderungen der Hautoberfläche. Arch Dermatol Forsch 248: 355–360
6. Schwarz E (1966) Freie Aminosäuren und Harnstoff in psoriatischen und normalen epidermalen Verhornungsprodukten. Arch Klin Exp Dermatol 225: 299–305
7. Schwarz E (1977) Neurodermitis und Hornschicht. Z Hautkr 52: 59–64
8. Wellner K, Fiedler G, Wohlrab W (1992) Untersuchungen zum Harnstoffgehalt der Hornschicht bei Neurodermitis. Z Hautkr 67: 648–650
9. Wheatley VR (1948) An improved diacetyl reaction for the estimation of urea in blood. Biochem J 43: 420–426
10. Wohlrab W, Hassler N (1981) Penetrationskinetik von Harnstoff in die menschliche Haut. Dermatol Monatsschr 167: 277–283
11. Zesch A, Hoffmann WD, Schaefer H (1974) Verteilung eines radioaktiv markierten Pharmakons in der menschlichen Hornschicht aus vier Salbengrundlagen. Pharmazie 29: 198–203