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# A COMPARATIVE STUDY OF THE PHYSICOCHEMICAL PROPERTIES OF HUMAN KERATINIZED TISSUES

HOWARD P. BADEN, LOWELL A. GOLDSMITH AND BARBARA FLEMING

*Department of Dermatology, Harvard Medical School and Massachusetts General Hospital, Boston, Mass. (U.S.A.)*

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## SUMMARY

Stratum corneum, hair and nail are all derived from ectodermal cells but show significant structural differences in their fully differentiated form. However, X-ray diffraction studies indicate that they all contain an $\alpha$-fibrous protein with the same molecular dimensions. The greater tensile strength and stability to heating of hair and nail compared to stratum corneum can be explained by their higher content of half cystine. Studies of the isolated structural proteins indicate that the appendages contain a non-helical matrix component very rich in cystine while stratum corneum does not. Furthermore, stratum corneum has an $\alpha$-fibrous protein with physicochemical properties quite different from those of hair and nail, which are very similar to one another. Despite different morphological characteristics, it appears that hair and nail have differentiated along very similar lines. However, subtle differences in the relative proportion and composition of the structural proteins can be detected. A distinguishing feature of stratum corneum is its large content of lipids which make it an effective barrier to the diffusion of water.

## INTRODUCTION

Keratinized tissues exhibit considerable heterogeneity in composition but they all contain structural proteins as major constituents[1,2]. In mammals these structural proteins are a mixture of $\alpha$-fibrous and globular proteins in varying proportions[3,4]. Studies of wool proteins have demonstrated considerable species variation in physicochemical properties, and in addition the fibrous and non-fibrous components themselves are heterogeneous[5]. No such data is available, however, on human hair, stratum corneum and nail plate.

A recent report on the harlequin fetus[6], a genetic disorder of keratinization, has indicated that there can be an abnormality of the fibrous proteins of epidermis without a similar change in hair. This suggests that the formation of fibrous proteins in the epidermis and appendages may be under the control of separate genes. One might anticipate, therefore, that differences could be observed in the fibrous protein

of different keratinized tissues and perhaps in their non-fibrous components as well. Crounse[7] compared the alkali-soluble proteins of hair, epidermis and nail and demonstrated striking similarities. The analyses were done on whole tissue, however, without prior separation of the different protein components. Development of newer techniques for the identification of the various structural proteins of keratinized tissue has made it possible to compare more precisely the proteins of epidermis, nail and hair. The purpose of this report is to compare the structural protein of human stratum corneum, nail and hair and relate these to the differences and similarities in the physical properties of these tissues.

## MATERIALS AND METHODS

All the chemicals used were of reagent grade except iodoacetic acid which was crystallized from anhydrous ether and light petroleum. Hair and nail clippings were obtained from normal individuals and washed with light petroleum before being used. Epidermis was separated from autopsy skin heated at 50 °C for 30 s and its undersurface was scraped with a scalpel to remove adherent epidermal cells. The remaining membrane consisted entirely of stratum corneum.

### *Extraction procedures*

The various tissues were homogenized in 20 vol. of 6 M urea in 0.1 M Tris, pH 9.0, (Tris–urea) and stirred at room temperature for 24 h. Following centrifugation the extraction was repeated a second time. The undissolved pellet was then extracted (10 mg/ml) at 50 °C for 2 h under nitrogen in Tris–urea with 0.1 M mercaptoethanol for stratum corneum and 0.2 M mercaptoethanol for hair and nail. The suspension was centrifuged and an aliquot of the supernatant treated with iodoacetic acid to give the *S*-carboxymethyl derivative[8]. We have determined that these conditions give complete reduction and blockage of cystine residues. The alkylated and remaining untreated extracts were then dialyzed against distilled water and lyophilized.

### *X-ray diffraction*

X-ray diffraction analysis was done using nickle-filtered copper $K_\alpha$ radiation ($\lambda = 1.54$ Å) at 40 kV at a specimen to film distance of 1.50 cm. Regenerated filaments for X-ray diffraction analysis were prepared by dissolving the protein in 80% formic acid, picking up the solution on the tip of forceps and stretching while drying at room temperature.

### *Stress-strain analysis*

Samples of hair were held between two sets of nylon clamps, one attached to a Stratham strain gauge (Stratham Lab., Los Angeles) and the other to a variable speed motor. The hair could be immersed in water or the whole apparatus placed in a chamber in which the humidity could be regulated. The specimen was stretched at constant rate and the tension recorded. Sonic velocity was measured by a dynamic modulus tester (L. M. Morgan Co., Cambridge, Mass.) as previously described[9]. The modulus of elasticity of nail was measured by deflection of the nail with different weights[10].

*Water holding*

The water holding capacity was determined by suspending specimens from calibrated quartz springs in glass chambers in a constant temperature bath held at 25 °C. The weight of the sample was determined by measuring the extension of the spring with a cathetometer. The humidity was controlled by placing anhydrous $CaSO_4$ or saturated solutions of the various salts in the bottom of the chambers[11].

*Water diffusion*

The diffusion of water was measured with aluminium chambers filled with water to which the specimen was fixed as a membrane. The chambers were stored with the specimen in contact with the water in desiccators at room temperature and weighed periodically to determine the rate of water loss.

*Amino acid analysis*

Samples for amino acid analysis were hydrolyzed in 6 M HCl for 24 h under vacuum at 110 °C and run in duplicate on a Beckman 116 amino acid analyzer.

*Electrophoresis*

Disc electrophoresis was done at pH 8.3 using a 7% acrylamide gel with and without 6 M urea[12].

*S content*

Total S content was determined gravimetrically following its oxidation to $SO_4^{2-}$ and the addition of Ba (Belmont Analytical Lab.).

## RESULTS

*X-ray diffraction*

The results of X-ray diffraction analysis of human hair, nail and stratum corneum are shown in Table I. Although the same reflections are observed in hair and nail, the reflections in the former are much sharper indicating a higher degree of orientation. Unstretched stratum corneum shows only unoriented halos when the X-ray beam is perpendicular to the surface of the specimen but a partially oriented $\alpha$ pattern when it is parallel. After stretching the specimen the $\alpha$ pattern shows much sharper reflections. The orientation of filaments is parallel to the growth axis in hair

TABLE I

WIDE ANGLE X-RAY DIFFRACTION REFLECTIONS OF STRATUM CORNEUM, HAIR AND NAIL

Very weak reflections were found at 4.15 Å and/or 4.39 Å (somewhat accentuated on the meridion) in some specimens of hair and nail and are likely due to contamination with soap[10].

| | *Equatorial reflection Å* | *Meridional reflections Å* |
|---|---|---|
| Stratum corneum (oriented) | 9.8 | 4.15, 5.14 |
| Nail | 9.8 | 5.14 |
| Hair | 9.8 | 5.14 |

and perpendicular in nail, while in epidermis there is only planar orientation unless the specimen is stretched. In epidermis an additional intense reflection can be seen at 4.15 Å which is meridional in position with stretched tissue. Treatment of the tissue with polar organic solvents removes this reflection and it has been shown the material producing it has the characteristics of a lipid[13].

*Water holding capacity*

The water holding capacity of stratum corneum, hair and nail at different relative humidities is shown in Fig. 1. Much more water is held by stratum corneum than by hair or nail at high humidity. The data at low humidity is less reliable because of difficulty in reaching equilibrium conditions. Washing the tissues with a chloroform–methanol mixture (3:1, v/v) followed by soaking in water markedly reduces the uptake of water by stratum corneum, but has no measurable effect on the other two tissues.

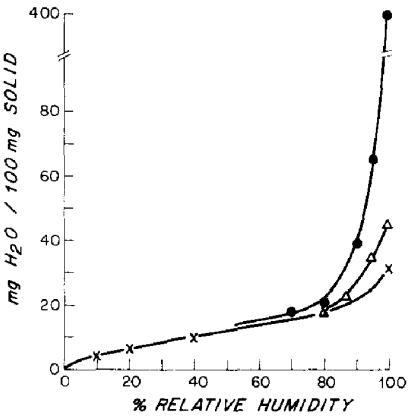


Fig. 1. Water content of stratum corneum, hair and nail at different relative humidities. ●, stratum corneum; △, stratum corneum extracted with a chloroform–methanol mixture; ×, nail and hair which gave identical results.

*Water diffusion*

The flux of water across stratum obtained from skin of the anterior abdominal wall is in the range 0.14 to 0.35 mg/cm² per h. Values almost ten times higher than this (2.0–3.0 mg/cm² per h) are observed for nail plate. Since the thickness of stratum corneum is about 1/100 that of nail, the diffusion constant of water is several hundred times greater for nail compared to stratum corneum. No effect of chloroform–methanol (3:1, v/v) extraction and soaking in water is observed for water flux in nail but the value for stratum corneum is increased almost 10-fold (1.9–3.2 mg/cm² per h) with this treatment.

*Response to heating in water*

The X-ray diffraction pattern of stratum corneum, nail and hair was determined before and after heating in water at 85 °C (ref. 14). No change is observed with hair and nail but with stratum corneum the pattern is lost and replaced by a cross $\beta$ one. Further heating of hair and nail produces no change until 130 °C when a poorly

oriented parallel $\beta$ pattern is noted in place of the normal $\alpha$ pattern. Isometric contraction studies of stratum corneum show a marked increase in tension starting at about 80 °C but no contraction is observed with nail and hair even when the heating is continued to 95 °C.

*Modulus of elasticity*

The modulus of elasticity can be determined by several techniques. The measurement of the modulus of elasticity by sonic velocity techniques permits a direct comparison of all three tissues and the modulus of elasticity for stratum corneum, hair and nail are shown in Table II. The latter two have a much higher modulus

TABLE II

YOUNG'S MODULUS FOR NAIL, HAIR AND STRATUM CORNEUM BY THE SONIC VELOCITY AND MECHANICAL TESTS

Some orientation of abdominal stratum corneum occurs during drying of the specimens.

| | *Number of specimens* | *Young modulus (dynes/cm² × 10⁻¹⁰ ± S.D.)* | | |
|---|---|---|---|---|
| | | *Sonic velocity technique* | *Mechanical stretching* | |
| | | *50% R.H.* | *100% R.H.* | *70% R.H.* |
| Nail | 12 | 4.3 ± 0.4 | 1.8 ± 0.5 | 2.6 ± 0.4 |
| Hair | 13 | 8.8 ± 0.6 | 1.5 ± 0.2 | 2.3 ± 0.3 |
| Stratum corneum | | | | |
| Abdominal | 2 | 1.2 ± 0.68 | 0.13 ± 0.07 | 0.19 ± 0.04 |
| Sole | 2 | 0.47 ± 0.15 | | |

than the former. This same difference is also observed when mechanical methods of measurement are used and these relative differences are also apparent at different degrees of hydration of the tissue. Extraction of the tissue with a polar organic solvent (chloroform–methanol, 3:1, v/v) does not alter the results indicating that the differences are related to the structural proteins and their intrinsic organization.

*Amino acid analysis of whole tissue*

The amino acid composition of hair, stratum corneum and nail is shown in Table III. Although both hair and nail show a much higher content of half cystine than epidermis, the value for hair is higher than that for nail. Analyses of total S confirm these differences in half cystine content.

*Extraction of proteins*

The solubility of tissue proteins was studied in hair, nail and stratum corneum by first extracting the tissue with Tris–urea and then with the same buffer with the addition of 0.1 M mercaptoethanol. The results are shown in Table IV and indicate that in all three tissues the addition of a reducing agent markedly increases the yield of protein. The Tris–urea buffer did extract more protein from stratum corneum, however, and about half of this remained soluble when the extract was dialyzed against a neutral salt solution. Electrophoretic patterns of the urea-soluble proteins

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