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# THE WATER-BINDING PROPERTIES OF THE WATER-SOLUBLE SUBSTANCES IN THE HORNY LAYER

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Studies by Blank (1), Jacobi (5), and others have shown that cornified epithelium becomes hard and brittle when it dries out. This can be remedied only by moisture; fats etc. do not help. Horny epithelium (callus) again becomes supple when it contains at least 10 mg moisture/100 mg dry weight (1). The stratum corneum receives a small amount of water by diffusion from the living epidermal layers, but when the relative humidity of the air is low, it releases more water than is supplied by diffusion and finally dries out. The relative humidity of the air is therefore the most important factor influencing the moisture content of the horny layer.

The capacity of stratum corneum to bind water depends mainly on the presence of substances easily soluble in water (2, 12). These substances are strongly hygroscopic *in vitro;* dried aqueous extracts of horny-layer material bind water several times their own weight when the relative humidity of the air is high (2, 9). Water-soluble substances can be removed from the horny layer more easily after the fats have been removed (2, 7, 11, 15).

It is not known exactly what substance (substances) may be responsible for the hygroscopic properties of the water-soluble fraction of the horny layer. Szakall (13) originally assumed that it was represented by free amino acids, but he was unable to prove this directly. Flesch and Esoda (3) found that psoriasis scales bind less water than normal horny-layer material and also contain less free amino acids. This reduced capacity to take up moisture was attributed by these authors to a decreased amino acid content. Later, Szakall (14) pointed to the occurrence of bound pentoses and hexoses in the form of Amadori and Maillard compounds, to which marked hygroscopic characteristics have been attributed. Spier and Pascher (9) and Spier *et al.* (11) believed, however, that other, unknown, hygroscopic substances must be present in the fraction extractable with water. In 1962 Spier and Schwarz ascribed the capacity of the water-soluble substances to take up water mainly to lactates and salts of 2-pyrrolidone-5-carboxylic acid (10). Fox *et al.* (4) also pointed to the hygroscopic properties of lactates, and Laden and Spitzer (6) referred again to the occurrence of the sodium salt of 2-pyrrolidone-5-carboxylic acid as hygroscopic substance in the horny layer.

It is clear from the foregoing that there is no general agreement on the question of just which water-soluble substances carry the hygroscopic properties of horny-layer extracts. The data in the literature have led some investigators to conclude that the free amino acids are mainly responsible for the hygroscopic properties of the water-soluble fraction, but the argumentation seems inadequate. We therefore determined the capacity of various water-soluble substances of the horny layer to bind water, both individually and in groups.

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Table 1. *Composition of a mixture in which all known water-soluble substances of the horny layer are present (based on data of Spier and Pascher, 1959). (The values listed relate to the amounts present in 100 g skin scrapings)*

| | | | |
|---|---|---|---|
| alanine | 754 mg | citric acid | 20 mg |
| arginine · HCl | 363 mg | creatinine (own addition) | 13 mg |
| aspartic acid | 648 mg | glucosamine | 26 mg |
| citrulline | 1285 mg | glucose | 215 mg |
| glutamic acid | 290 mg | glycogen | 18 mg |
| glycocoll | 870 mg | 1 N KOH | 10.98 ml |
| histidine · HCl | 860 mg | lactic acid | 1606 mg |
| leucine | 416 mg | formic acid | 0.03 ml |
| lysine · HCl | 232 mg | $MgCl_2 \cdot 6\,H_2O$ | 226 mg |
| ornithine · HCl | 255 mg | $Na_2HPO_4 \cdot 2\,H_2O$ | 63 mg |
| phenylalanine | 193 mg | 1 N NaOH | 23.3 ml |
| proline | 203 mg | $NH_4Cl$ | 107 mg |
| serine | 2515 mg | 2-pyrrolidone-5-carboxylic acid | 1840 mg |
| threonine | 512 mg | ribose | 50 mg |
| tryptophane | 106 mg | urea | 550 mg |
| tyrosine | 309 mg | uric acid | 30 mg |
| valine | 280 mg | urocanic acid | 560 mg |
| $CaCl_2 \cdot 2\,H_2O$ | 942 mg | | |

## Material and Methods

Our study was undertaken in light of the data of Spier and Pascher (9), who collected skin scrapings (peripheral part of the stratum corneum dysjunctum) of healthy, non-sweating subjects for analysis of the water-soluble fraction, of which about 5–10 % has not yet been identified. On the basis of their analytical results they composed a mixture containing all the known water-soluble substances in physiological proportions (see Table 1).

In our study the water-binding capacity of all the free amino acids occurring in the horny layer was first determined individually. On the basis of Spier and Pascher's data, physiological mixtures were then made of all amino acids (a), of all the other substances (b), and of all known horny-layer substances combined (c). The water-binding capacity of all these mixtures was also compared with complete horny-layer extracts.

Samples (±50 mg) of the individual amino acids and the mixtures were placed in weighing flasks of known weight. Complete horny-layer extracts were obtained as follows: In 12 non-sweating subjects with normal skin, the peripheral part of the stratum corneum dysjunctum was scraped from the fore-arms with a scalpel. These scrapings were pooled (total quantity about 1000 mg), divided into 3 portions, washed 3 times with ether, and extracted for 48 hours with 15 ml distilled water at 4°C. After being passed through filter paper, the extracts were brought into weighing flasks. This way of collecting and processing a large quantity of scrapings was laborious and later on the method of Spier and Schwarz (10) was adopted. Both fore-arms of 10 non-sweating subjects with normal skin were "de-fatted" with a wad of cotton dipped in ether and then held for 2 minutes in 3 litres of water with a temperature of about 20°C. The resulting extract was processed in a vacuum rotation evaporator[1] at 40°C until the volume was reduced to a few millilitres, after which the residue was divided into a number of portions and brought into weighing flasks.

All the mixtures and skin extracts were then dried in a drying oven at 37°C, and placed in exsiccators above $P_2O_5$ for 4 days for further drying to a constant weight. The initial quantities were chosen in such

[1] Rotovapor R, Büchi.

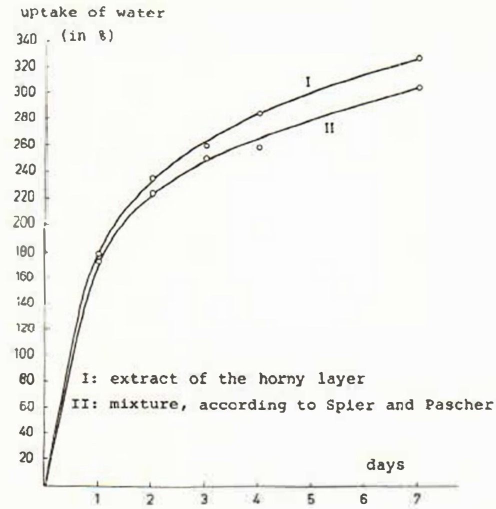


Fig. 1. Uptake of water by a natural extract of the horny layer (I) and by a synthetic mixture of water-soluble substances (II) according to Spier and Pascher.

a way that the dry weight of all samples was about 50 mg.[2] The $P_2O_5$ was then replaced by a mixture of 10 % $H_2SO_4$ in water, which at room temperature (about 20°C) gives a relative humidity of 95 %. After a period of 7 days, the exsiccators were opened.[3] The flasks were sealed and weighed.[4] The quantity of moisture taken up under the experimental conditions was calculated. The results were expressed as percentage of dry weight (Tables 2 and 3). In addition, 2 samples (a mixture of all the known substances and an extract of scrapings) were weighed daily to determine the increase in weight (Fig. 1).

## Discussion

It is evident from Table 2 that of the individual amino acids only the basic amino acids ornithine, lysine, and arginine are capable of binding several times their own weight of water under the experimental conditions given. The calculated amount of water that an amino-acid mixture (when these amino acids occur in correct proportions) is capable of binding is about 30 per cent. But it will be seen from Table 3 that a mixture of all 17 amino acids can take up many times more water than would be expected from the water-binding capacity of the individual amino acids (268 %). We are unable to explain this phenomenon. It may possibly be due to salt formation between the amino acids.

Horny-layer extracts contain 40 to 50 per cent amino acids. If the water-binding capacity of these extracts depended solely on the presence of the mixture of amino acids, they would be able to bind only 110 to 130 per cent which is only about a third of the observed capacity. Mixtures of the other known components (b) of the water-soluble fraction proved to be even more strongly hygroscopic than the amino-acid mixture.[5] If all the known small-molecular substances of the horny layer are combined, the resulting mixtures can take up 288 per cent water on the average. Such mixtures represent about 95 per cent of all the water-soluble substances. Consequently, horny-layer extracts should be able to take up 275 per cent water. The water-binding capacity of complete natural extracts was about 310 per cent; this can be explained almost entirely on the basis of the known small-molecular substances in these extracts.

It is not certain whether the unknown residue of the water-soluble substances also has components contributing an additional effect (separately or together with the known substances) to the hygroscopic characteristics of the water-soluble substances. This question is also difficult to answer because it is not certain that the composition

[2] To obtain reproducible and comparable results, the same quantities of the various substances (or mixtures of substances) must be used throughout.

[3] Although equilibrium had not been reached at the end of this period, a preliminary study had shown that it was not reached even after 3 weeks.

[4] Mikrowa balance, type AW10, accurate to within 0.2 mg.

[5] Na-lactate and the Na-salt of 2-pyrrolidone-5-carboxylic acid could bind large quantities of water (about 400 % and 350 %, respectively). The other substances in this mixture were not investigated separately.

Table 2. *Water-binding capacity of individual amino acids of the horny layer (% of dry weight; relative humidity 95 %)*[*]

| | Range | | Range |
|---|---|---|---|
| alanine | 1 (0–2) | ornithine · HCl | 225 (195–290) |
| arginine · HCl | 142 (132–163) | phenylalanine | 0 (0–0) |
| aspartic acid | 1 (0–1) | proline | 2 (0–4) |
| citrulline | 11 (8–18) | serine | 1 (0–2) |
| glutamic acid | 0 (0–0) | threonine | 1 (0–2) |
| glycocoll | 7 (6–8) | tryptophane | 2 (1–2) |
| histidine · HCl | 2 (0–4) | tyrosine | 1 (0–2) |
| leucine | 1 (0–2) | valine | 2 (1–2) |
| lysine · HCl | 243 (222–260) | | |

[*] Values are means of 5 determinations.

Table 3. *Water-binding capacity of mixtures of various substances of the horny layer (% of dry weight; relative humidity 95 %)*

| Synthetic mixture of amino acids in proportions present in the horny layer (a) | Synthetic mixture of all other known water-soluble substances of the horny layer (b) | Mixture of all known substances of the horny layer in physiological proportions (c) | Natural extract of the horny layer |
|---|---|---|---|
| pH[*] = 5.58 | pH[*] = 4.50 | pH[*] = 5.20 | pH[*] = 5.25 |
| 262 | 413 | 292 | 316[**] |
| 290 | 401 | 267 | 300[**] |
| 267 | 401 | 300 | 306[**] |
| 262 | 404 | 274 | 295[***] |
| 260 | 400 | 307 | 330[***] |
| 268 | 404 | 288 | 309 |

[*] Measured at the end of the experiment (after uptake of water).
[**] Extract of skin scrapings.
[***] Extracted *in vivo*.

of the mixture of substances given in Spier and Pascher's Table (Table 1) is identical to that of our extracts. But Spier and Pascher's model certainly represents a good approach to the composition of complete extracts, as confirmed by the fact that the amino-nitrogen content of our scraping extracts amounted to 6.7 per cent, which is in near complete agreement with the model.

It is clear from the foregoing that the amino acids( both individually and as mixtures) have limited importance for the water-binding capacity of horny-layer extracts. On the other hand, there is no reason to ascribe the hygroscopic properties of the water-soluble fraction of the horny layer mainly to the unknown residue (5–10 %), as proposed by Spier and Pascher (9) and Spier *et al.* (11). The water-binding capacity of a mixture of all known water-soluble substances approaches the water-binding capacity of complete horny-layer extracts rather well. Although sodium-pyrrolidone-carboxylate and sodium-lactate have proved to be strongly hygroscopic, we do not, on the basis of calculation, agree with the opinion of Spier and Schwarz (10) that the water-binding capacity of the water-soluble substances of the horny layer should be ascribed almost completely to these salts. The hygroscopic characteristics of the water-soluble fraction of the horny layer are the result of the presence of numerous water-soluble substances together. This conclusion accords with the most recent opinion expressed by Spier (8) concerning this problem, although no reasons are given for his change of views.

## SUMMARY

Data in the literature indicate that the water-binding capacity of the horny layer is dependent mainly on the presence of small-molecular water-soluble substances. Horny-layer extracts are strongly hygroscopic *in vitro*, but it is not certain what substance or substances are responsible. From the results of the present study of the water-binding capacities of various groups of water-soluble substances it is concluded that the hygroscopic properties of the water-soluble fraction of the horny layer must be attributed to the simultaneous presence of a large number of different substances. There appears to be no reason to attribute these hygroscopic properties mainly to the small residue that has not yet been adequately analysed.

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