

Water and Ion Distribution Profiles in Human Skin

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Bulk sample x-ray microanalysis (XRMA) of human gluteal skin was performed to provide data on water and ion profiles over the epidermal cross section. All samples were analyzed both in the frozen hydrated state and in the frozen dried state. This allows, for the first time, a quantitative determination of the local water content in different strata of the skin. A steep water gradient was found in the stratum corneum towards the stratum granulosum, while the water content was constant in all deeper layers of the skin, including the papillary dermis. Previously demonstrated distributions over the epidermis of the monovalent ions sodium (Na), potassium (K) and chlorine (Cl) given in concentrations per unit dry weight were confirmed, as were the distributions of phosphorus (P) and sulfur (S). Combining the water and ion distributions, our data indicate the major monovalent ions Na, K and Cl to be in equilibrium between the stratum corneum and the dermis if the assumption is that all monovalent ions are in solution and that all the water of the tissue is available as solvent for the ions. This result does not disprove the existence of an ion transport gradient in the epidermis but sets significant bounds to it.

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The aim of this paper was to shed light on the role of water and ion distribution in skin physiology because, in order to understand the transport and barrier functions of the skin, it is important to have knowledge of the ion and water distribution of the different strata.

X-ray microanalysis (XRMA) of cryoprepared specimens is the method of choice for the measurement of local ion and water concentrations in biological samples (1,2). XRMA of freeze-dried cryosections was introduced in dermatology in 1982 (3-5), when it was used to measure the distribution of elements across guinea-pig as well as human skin (6). The main emphasis on elemental analysis in skin has been laid on the effects caused by known irritants and allergens (7). It has been possible to demonstrate dose and time dependency in the cellular reactions, spanning from signs of cell stimulation of replication to signs of cell damage as inferred from elemental dry weight data. However, data on the distribution of water in skin are still scarce. Attempts to solve this problem with XRMA were made by Warner et al. (8,9). In order to obtain a fully quantitative estimate of the water distribution in the skin, we decided to examine bulk sections from cryofractured skin biopsies by XRMA in the frozen hydrated as well as the frozen dried state. This allows the examination of element distribution profiles per local dry mass and per compartment water as well as the calculation of the local dry mass or water distribution (10-12).

MATERIAL AND METHODS

Three 3-mm punch biopsies each from 3 healthy persons (2 women, one man) were taken from the gluteal region. In one case (the male) local anesthesia with Xylocaine without adrenaline was used. The samples were immediately snap frozen and subsequently stored under liquid nitrogen until analysis. They were mounted onto squeeze-type aluminum stubs and transferred under liquid nitrogen to the cold stage of a Polaron E7400 cryopreparation chamber fitted to a Phillips 525 scanning electron microscope. At about 100 K the samples were cryofractured perpendicularly using a blunt-ended rod and then sputter-coated with a thin gold layer in order to decrease charging effects (13). This allows identification of the different strata even in frozen hydrated samples (Fig. 1).

The samples were first analysed in the frozen hydrated state at about 150-170 K (10). After freeze-drying for at least 60 min at 213 K, the samples were analysed in the freeze-dried state at 150 K. Using a LINK AN10,000 microanalyser, analysis was carried out at an accelerating voltage of 20 kV, in the following strata: stratum corneum, stratum spinosum, stratum basale, upper dermis (papillary layer) deep dermis (about 100 µm below the epidermis). The size of the measuring spot was determined by a rectangular raster to about 5×5 µm² (stratum corneum) or 10×10 µm² (other strata). Between 5 and 10 measurements were performed in each stratum per specimen, yielding a total of 30 measurements per subject in most of the strata. The live time of analysis was 100 s and the total time of processing per biopsy was about 8 h. In subject no. 1 (the male subject), however, only two specimens could be measured, resulting in a number of 13 measurements per stratum in this case. Moreover, in the biopsies from this subject, the stratum corneum could not be identified unambiguously, and no measurements were performed in this layer.

Quantitative evaluation of spectra was performed using ZAF-PB software (1,14). Thick frozen sections of a gelatine solution containing one of the salts Na₂SO₄, MgSO₄, KH₂PO₄, KCl, or CaCl₂ in known concentrations in the range from 50 to 400 mmol/kg dry weight were used as standards (15).

The procedure gave concentrations of elements per kg wet weight (C_{wet}) or per kg dry weight (C_{dry}), respectively. The local dry mass fraction F (dry mass per wet mass) was calculated according to (1)

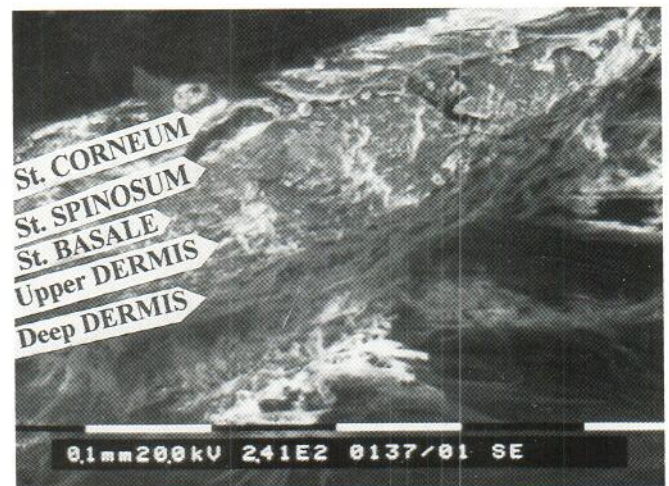


Fig. 1. Cross-section through freeze-fractured human skin. White bar denotes 100 µm.

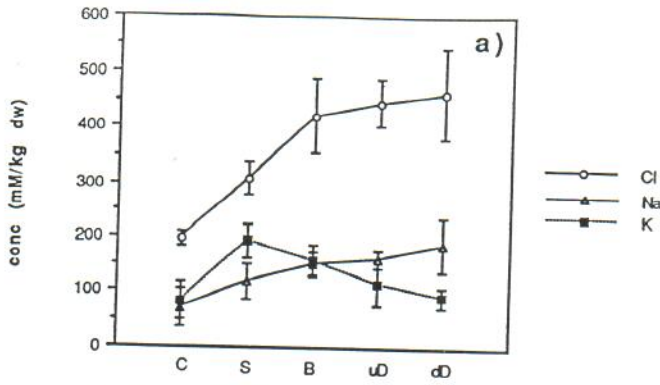


Fig. 2a. Elemental distribution of sodium, chlorine and potassium calculated as millimoles per kg dry weight. Data are given as mean \pm SEM. C: stratum corneum; S: stratum spinosum; B: stratum basale; uD: upper (papillary) dermis; dD: deep (reticular) dermis.

$$F = C_{wet}/C_{dry} \quad (1)$$

using the Cl concentration values, because Cl was the most prominent and unobstructed peak in the spectrum from the frozen hydrated samples.

Assuming that all the ions are dissolved in the tissue water, concentrations of ions per litre water C_w can be calculated as:

$$C_w = C_{dry} * F/(1-F) \quad (2)$$

Standard errors of means (SEM) were calculated according to the Gaussian law of error propagation. Means were compared by analysis of variance and the critical difference between means (CDM) was calculated at the 5% significance level. Mean values differing by more than the CDM were taken as significantly different.

RESULTS

The structure of the cryofractured skin is shown at low magnification in Fig. 1. The fractured surface is rather rough, especially in the fibrous dermis, facilitating the identification of the different strata.

The distribution of the elements Na, Cl, K, Mg, P, and S, calculated as concentrations per unit of dry weight, is given in Fig. 2. Despite interindividual variances, there were clear gradients across the skin: Na and Cl concentrations per dry mass increase from the stratum corneum to the dermis. Mg concentrations were high in the stratum corneum but constantly low in all other strata. This is different from Ca concentrations,

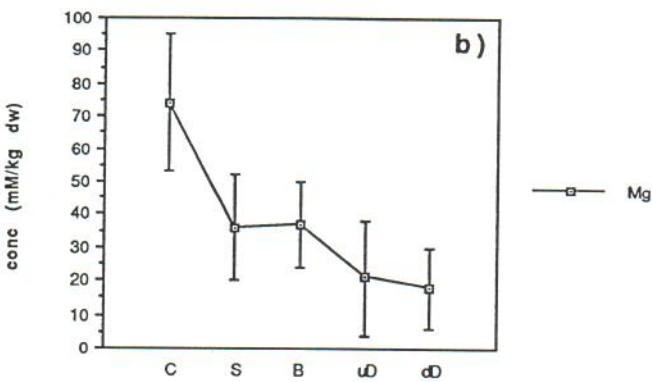


Fig. 2b. Distribution of magnesium calculated as millimoles per kg dry weight. Data are given as mean \pm S.E.M. Abbreviations as in Fig. 2a.

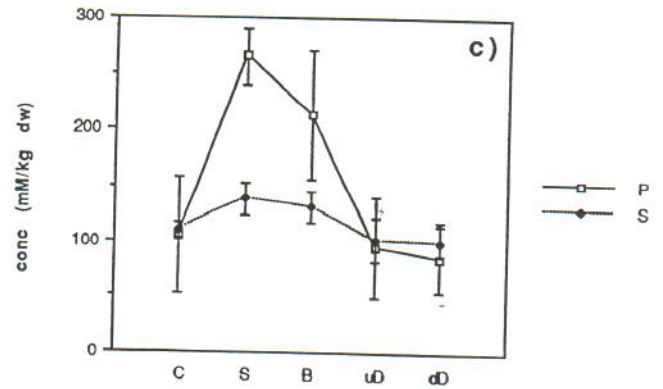


Fig. 2c. Distribution of phosphorus and sulfur calculated as millimoles per kg dry weight. Data are given as mean \pm S.E.M. Abbreviations as in Fig. 2a.

consistently around 5 mmol/kg dry weight in all strata measured, which is just above the threshold of sensitivity (data not shown). Averaged over all 3 individuals, K and P concentrations were low in the stratum corneum, high in the stratum spinosum, and low again in the dermis. The sulfur values followed this distribution, although less exaggerated. This pattern is well in accord with earlier observations in guinea-pig as well as in human skin (16). However, only weak gradients of K, P, and S were measured in biopsies from subject no. 1.

Fig. 3 shows that the local water content was rather uniform in the different strata, with the sole exception of the significantly less hydrated stratum corneum. However, the ion distribution across human skin, expressed in millimolar concentrations of Na, K and Cl (Fig. 4) appears quite different from the same elemental distribution if expressed as ion concentrations per dry weight (cf. Fig. 2). Assuming that the ions are completely dissolved in the skin water phase, Fig. 4 shows that the Na and Cl concentrations are the same in the stratum corneum and the dermis, while there is a rather weak, negative gradient of K from the outside of the skin towards the dermis.

DISCUSSION

Using x-ray microanalysis there are a number of methods to measure the local water content in biological tissues (11). We prefer fully quantitative x-ray microanalysis of both hydrated and freeze-dried samples (11, 14). This technique requires no additional assumptions. It is insensitive to possible artifacts

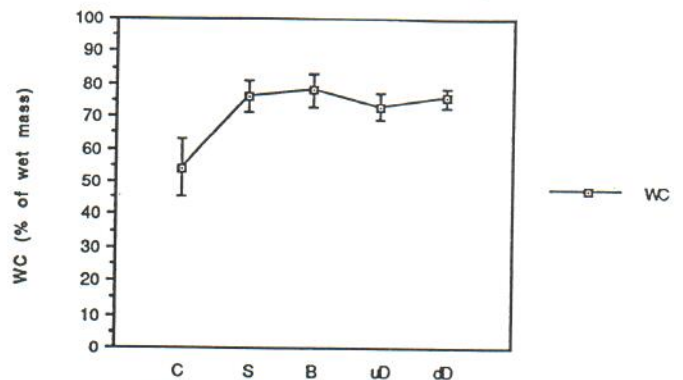


Fig. 3. Water concentration in per cent of wet mass. Data are given as mean \pm S.E.M. Abbreviations as in Fig. 2a.

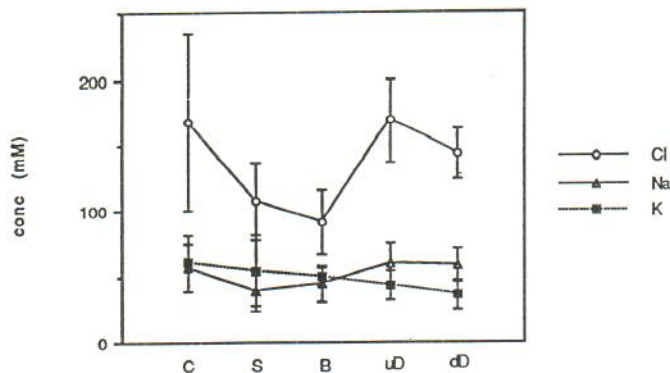


Fig. 4. Distribution of sodium, chlorine and potassium concentrations in tissue water of the human skin cross-section. Data are given as mean \pm S.E.M. Abbreviations as in Fig. 2a.

during tissue preparation and against radiation damage during the measurements.

The present study has yielded three major results:

Firstly, the elemental distribution per dry mass as measured here by bulk specimen x-ray microanalysis essentially confirms earlier data obtained on thick sections of both guinea-pig and of human skin (16) and those obtained on thin sections of human skin (8, 9). As discussed by Forslind et al. (6), high P concentrations per dry mass in the stratum spinosum and basale are expected due to the high concentration of phospholipids and nucleic acids in these strata composed mainly of metabolically active cells. Moreover, K, as an intracellular cation, is expected to follow the same distribution pattern, while Na and Cl should be highest in the dermis which is essentially an extracellular compartment. It should be noted that we obtained low concentrations per dry mass for all monovalent ions in the stratum corneum in contradiction to the results obtained by Warner et al. (8).

Secondly, our study confirms the qualitative distribution of water across the human skin as presented by Warner et al. (9). In addition, it shows that the hydration of the dermis is not significantly different from that of the viable cell layers of the epidermis. However, our fully quantitative analysis results in a somewhat higher water content in the viable strata than that given by Warner et al. (9). This difference is probably due to the averaging over cells and extracellular spaces in our study.

The average water content of the stratum corneum as measured in this study is 54%, while Warner et al. (9) arrived at a water content as low as 15 to 40% in the same layer.

Thirdly, this study provides for the first time quantitative estimations of the ion concentrations per compartment water, i.e. millimolar ion concentrations, in the different strata of human skin. In interpreting these data, one has to bear in mind that the values measured in the stratum basale and stratum spinosum represent averages between the intra- and extracellular compartments due to the probe size. However, the important result is that there are no gradients of Na and Cl and only a weak gradient of K between the stratum corneum and the dermis as demonstrated in our study. The Mg concentration, on the other hand, is significantly higher in the stratum corneum as compared to all other strata.

With respect to stratum corneum, our data appear contra-

dictory to those obtained by Warner et al. (8). These authors found steep gradients of monovalent ions (which will be exaggerated if calculated as millimolar concentrations) and water over the stratum corneum and even steeper water gradients between the stratum corneum and the stratum spinosum. Although the resolution of bulk specimen x-ray microanalysis is too low to scan water gradients within the stratum corneum, our results with rather small standard errors clearly exclude any significant ionic gradients within the stratum corneum. Moreover, these results demonstrate the Na and Cl ions to be in equilibrium across the skin, provided that they are free in solution. This is reasonable as a first approximation taking into account that the stratum corneum is composed of dead cells, and that the intercellular spaces in the stratum spinosum and basale have not been excluded from the measurements. In addition, the dermis is essentially an extracellular compartment. The decrease of Na and Cl in the stratum spinosum and the stratum basale is of course due to exclusion of these ions from the intracellular spaces.

The K distribution might have a somewhat different background. The fact that the K concentrations in the layers of the skin rich in metabolizing cells are not higher than those in the stratum corneum and dermis speaks in favour of a K binding in those essentially extracellular strata. Evidence for compartmentalization of monovalent ions, especially K, with extracellular mucoid substances (17) as well as with intracellular polyelectrolytes (cf. review 12) has been presented. It is not clear, therefore, whether the weak K gradient seen in Fig. 4 reflects any actual gradient of K in solution, which would then be indicative of a K transport. More likely, free K concentrations in stratum corneum and dermis might be in equilibrium with rather high fractions of K bound in both layers. This, of course, does not exclude local recycling of K.

Taken together our results indicate a gross ionic and osmotic equilibrium between the stratum corneum and the deep layers of the skin, if we accept that all monovalent ions are in solution. It is, however, conceivable that a certain fraction of monovalent ions in fact is bound to the polymers in the skin. Unfortunately x-ray microanalysis cannot resolve this problem, as it quantitates the elements regardless of their chemical status. The gradients of Na and Cl between the stratum corneum and spinosum can be explained solely by taking into account the exclusion of these ions from the metabolizing cells in the stratum spinosum. In other words, we have not found evidence for an active ion transport from the stratum corneum and spinosum towards the dermis in the skin of 3 healthy human subjects analyzed. This result, of course, does not disprove the existence of such an ion transport, considering the possibility that a certain fraction of ions is bound to polyelectrolytes of the epidermis. Further studies are required to clarify this point.

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