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BOUND WATER IN STRATUM CORNEUM MEASURED BY DIFFERENTIAL SCANNING CALORIMETRY*

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ABSTRACT

Differential scanning calorimetry has been used to study water binding in stratum corneum. Samples of various total water contents are frozen and melted and the latent heat of melting measured with a Perkin Elmer differential scanning calorimeter. From this the relative amounts of freezing (unbound) water and non-freezing (bound) water are calculated. The results show that the amount of bound water in human limb corneum is about 0.34 grams per gram of dry corneum. This supports the findings of Hansen and Yellin, based on NMR measurements, of 0.3–0.35 grams of bound water per gram of dry corneum. It indicates also that the 5.0 grams of bound water per gram of dry corneum which Scheuplein and Morgan estimated from rate of weight loss measurements refers to liquid water held in the tissue by diffusion barriers and not to water molecularly bound to other components of the stratum corneum. The effect of extracting lipids and water soluble substances from guinea pig footpad corneum is to increase the amount of bound water it can contain from 0.29 to 0.41 grams per gram of dry corneum.

The importance of the role of water in plasticising stratum corneum has been known since the classic work of Blank (1). It is reasonable to suppose that the water is acting on the keratin components which comprise some 60% of human corneum but the exact mechanism by which water brings about the plasticising effect is unknown. Knowledge of the mode of binding of water in corneum is obviously important in understanding this process. Many of the measurements of "water binding" in corneum that exist in the literature (3, 4) are in fact simply measurements of the "water-holding" capacity calculated by direct weighing. Few workers have attempted to differentiate between water in corneum which is chemically or physically "bound" and that which exists as "free" or bulk water.

Using differential thermal analysis (DTA), Bulgin and Vinson (5) showed that in addition to a free-water fraction in human corneum and neonatal rat corneum there are two separate bound-water fractions. These measurements were qualitative only but there are also two widely differing estimates by Scheuplein and Morgan (6) and Hansen and Yellin (7) of the amount of bound water which exists in human corneum. The former, by measuring desorption rates of water, have stated that human corneum can absorb up to five times its own weight of water as "bound" water. The latter, by NMR and infra-red spectroscopy, estimated a bound-water fraction equivalent to 0.30–0.35 grams per gram of dry corneum.

The measurements to be described here tend to support those of Hansen and Yellin and an alternative explanation is offered to account for the observations of Scheuplein and Morgan. The technique of differential scanning calorimetry (DSC) has been used to measure the relative amounts of freezing and non-freezing water in corneum. Kuntz *et al.* (8) have shown for protein and nucleic acid solutions that the freezing fraction may be associated with "free" water and the non-freezing fraction with "bound" water.

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MATERIALS AND METHODS

The instrument used was a Perkin Elmer DSC-1, which was calibrated for measuring heats of transition using both benzene and water samples. Guinea pig footpad corneum was used as the main experimental material, being readily available, but the results were compared with those for human corneum obtained from amputated limbs, (not plantar corneum). The samples were prepared by the method described by Middleton (4). The purity of the samples was checked by histological examination.

The dry weight of each of the samples (typically 2–3 mg) was determined after drying over molecular sieve. They were then hydrated in atmospheres of varying relative humidities (obtained by saturated salt solutions) and then sealed hermetically in aluminium capsules and reweighed to find the total water uptake. The samples were then transferred to the calorimeter and cooled to 250° K before being heated at a controlled rate (8° K/min) to 290° K. The thermal transitions of the samples were registered on a chart recorder.

RESULTS

Figure 1 shows a typical series of traces obtained from the chart recorder for guinea pig footpad samples having different total water contents. In the case where the water content was below about 0.30 grams per gram of dry corneum a completely flat trace was obtained. When the water content was slightly higher than 0.3 g/g, a small endothermic (ice-melting) peak was recorded at 273° K. For even higher water contents

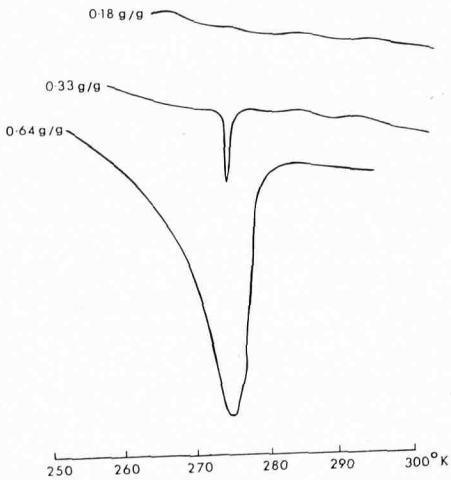

FIG. 1. D.S.C. traces for untreated guinea pig footpad with various water contents.

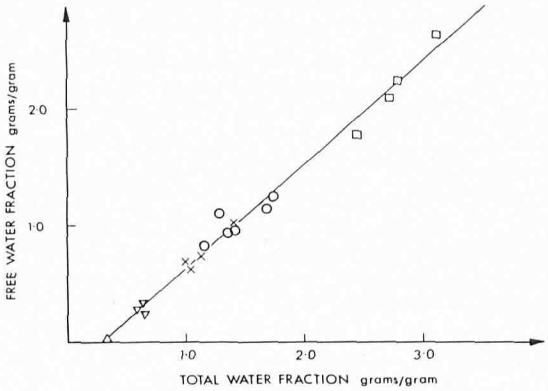

FIG. 2. The variation of free water fraction with total water fraction for untreated guinea pig footpad corneum.

the magnitude of the peak area (proportional to the heat absorbed in the transition) increased in proportion to the mass of the water present.

The heat absorbed in the transition was calculated in each case from the areas under the peaks and the equivalent mass of "free" water found by dividing by the latent heat of the ice-water transition. Figure 2 shows a plot of the free water quantity in guinea pig footpad corneum against the total water quantity in the corneum, each expressed in grams per gram of dry corneum. It is fairly evident that there is a linear relationship.

The intercept of the straight-line fit in Figure 2 on the total-water axis is at 0.29 g/g. This effectively represents the maximum quantity of bound (non-freezing) water that may be absorbed by dry corneum before the appearance of free (freezing) water.

A linear plot similar to Figure 2 is obtained for guinea pig footpad in which the lipids and water-soluble substance have been extracted with ether followed by water: the intercept is 0.41 g/g. Human limb corneum behaves similarly to guinea pig footpad corneum and traces like those of Figure 1 are obtained for samples of varying water contents. However, it was not found possible to attain such high values of hydration. The results for human limb corneum are shown in Figure 3. An intercept at 0.34 g/g is obtained.

It might be expected that, after the initial binding sites (represented by the intercept) had been satisfied, further water entering the corneum would remain as free water, in which case, the slope of the straight-line fit would be unity. For extracted corneum the slope is 1.06, which is not significantly different from unity. For untreated guinea pig corneum the slope is 0.89. However, it has been shown (10) that when dissolved substances are present, as will be the case for the unextracted samples, instrument limitations cause measured heats to be less than the true heats. A method of correcting for this effect has been suggested (10). When this correction is made a slope of 1.05 is obtained which again is not significantly different from unity. (The value of the intercept is not changed.) For human corneum the calculated slope (0.79) of the straight-line fit to the plot (Fig. 3) of free water against total water has a large error (±0.18) brought about by the limited range of values which may be obtained. Here also a correction for the effect of dissolved substances should be applied and again there is no significant difference between the value of the slope and unity.

The NMR measurements on human corneum of Hansen and Yellin (7) demonstrated the existence of two water fractions having different molecular mobilities—the time constants of the two fractions differing by a factor of about six. Both fractions were between an "ice-like" and a "bulk-liquid-like" state. Also infra-red measurements carried out at the same time on partially deuterated human corneum at low temperatures showed the existence of two components: a broad Gaussian band at 2472 cm^{-1} attributed to liquid water, and a sharp Lorentzian band at 2440 cm^{-1} attributed to ice. Both the NMR and IR data indicated that 0.30–0.35 cm^3 of water per gram of dry cor-

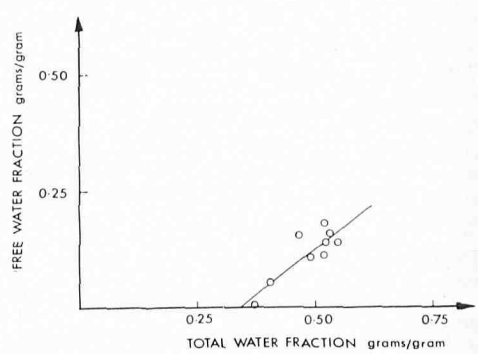

FIG. 3. The variation of free water fraction with total water fraction for untreated human corneum.

neum was in the less mobile state. The respective values of 0.20 g/g and 0.34 g/g for the bound-water fraction obtained from the intercepts in Figures 2 and 3 obviously compare well with the value given by Hansen and Yellin for the less mobile water fraction.

Scheuplein and Morgan (6), by observing the water desorption rate of hydrated human corneum, reported that after the initial rapid loss of surface water there is a distinct decrease in the rate of desorption. They attribute this to the desorption of "bound" water and state that this can be as much as five times the dry weight of the tissue. However, the results described here and those of Hansen and Yellin indicate that most of what Scheuplein and Morgan refer to as "bound" water must in fact be in the normal liquid state and not bound molecularly to other components of the stratum corneum. An alternative explanation for the transition in the desorption rate is that most of the water desorbing is intracellular water which is diffusing slowly through the cellular membrane, i.e., the desorption rate is controlled by diffusion through barriers.

Scheuplein and Morgan also noted a further transition on the desorption curve which they attributed to the 5–10% of "water strongly bound to the polar groups of the side chains of keratin." It is not unreasonable to suppose that this fraction (although apparently much smaller) may correspond to the bound water represented by the intercepts in Figures 2 and 3 since Scheuplein and Morgan state that their method of estimating the bound water fraction is arbitrary and tends to give an underestimate.

The DTA results of Bulgin and Vinson (5) are more difficult to relate since they are purely qualitative. However, of the three fractions, which are desorbed at 103° C, 114° C and 135° C, the first is obviously free water. Of the remaining two fractions, that desorbed at 114° C can be removed by drying the corneum over drying agent whereas that at 135° C may not. The first of these would therefore, seem to correspond to bound (non-freezing) water as measured by the DSC experiments since only that water removable by molecular sieve is taken into account in these experiments. The remaining fraction absorbed at 135° C would seem therefore to correspond to water very tightly bound within the corneum and not measurable by this technique.

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