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Equilibrium water sorption characteristics of the human nail

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Synopsis

The physical and transport properties of keratinized tissues are closely related to their water content. This report presents water uptake and desorption isotherms for the human nail and compares them with those of wool, horn, hair, and stratum corneum. Nail absorbed a maximum of ~0.3 g H_2O/g dry tissue, with the shape and magnitude of the isotherm most closely resembling horn. Hysteresis between uptake and desorption was observed, similar to that of other keratins. The shape of the isotherms was adequately described by both the D'Arcy-Watt and Guggenheim-Anderson-deBoer (GAB) models; however, small positive deviations from both models were found in the relative humidity range, 30–60%. Directionally better fits to the data were found with the D'Arcy-Watt model. This analysis suggests that most of the water in the tissue was in a strongly bound state, consistent with observations made by other techniques.

INTRODUCTION

Transport of small molecules through hydrophilic polymer matrices is known to depend sensitively on the hydration state of the polymer (1–3). In low-swelling systems, where a continuous water phase does not exist, this dependence can most easily be related to an increase in segmental mobility (1) and/or free volume (2,3) within the polymer matrix as it is plasticized by water. Binding of the solute to the polymer fibers can also play a role in transport (4). These effects can readily be seen in keratin–water systems, where transport of water itself has been particularly well studied. Water diffusivity in wool (5–8), horn (9), and the corneocyte phase of stratum corneum (4) increases enormously with increasing water content in the tissue. This phenomenon can be understood on the basis of free volume theory (2–4). A key element of applying such theories is that the water content of the tissue be accurately known.

In stratum corneum it is well known that permeability to solutes other than water is increased by hydration (10–13). The influence of water on nail permeability is much less certain. In fact, the equilibrium water sorption characteristics of human nail are not well established. In light of renewed interest in ungual drug delivery (14–16) and the possibility that delivery rates may be modified by hydration, we have undertaken a study

of this phenomenon. This report presents equilibrium water sorption and desorption isotherms for human nail, as well as interpretation thereof in terms of isotherm models. Related reports will describe the effects of hydration on water diffusivity in nail and on the permeability of nail to an antifungal drug, ketoconazole.

Equilibrium water sorption in nails from mixed mammalian species has been reported by Baden (17). He found a maximum water uptake of 0.3 g H_2O/g dry nail in studies conducted at 26°C. No hysteresis between uptake and desorption curves was noted. These data are discussed and quantitatively analyzed in this report, along with our own observations. Comparisons are also made to equilibrium water uptake in other keratinized tissues.

MATERIALS AND METHODS

Frozen intact cadaver nails were obtained from ScienceCare Anatomical (Phoenix, AZ). Human nail clippings were collected from several donors in our facility. Demographic information for each nail sample (nail clippings and intact cadaver finger and toe nails) was obtained. Nail samples were washed with a mild liquid detergent (containing sodium laureth sulfate and cocamidopropyl betaine) and dried at 45°C to a constant weight (Mettler AE 100). Intact cadaver nails from three different donors were used with n = 4–6 nails per donor. Their average dry weight ranged from 0.08 g (for little finger nails) to 0.50 g (for big toe nails). Nail clippings from multiple donors were pooled, then divided into six samples ranging from 0.26–0.40 g dry weight. In the analysis they were considered as from one donor, with n = 6. The water-binding capacity of nail samples was determined by transferring individual nail samples to a weighing dish and exposing samples to the vapor phase of solutions of varying relative humidity (RH) in a glass chamber maintained at 32°C. RH ranging from 11% to 100% was maintained with various concentrations of H_2SO_4, NaCl, K_2CO_3, and LiCl in water. Standard tabulations of molal osmotic coefficients φ over a range of temperatures were interpolated to 32°C, then converted to RH using the relationship $RH = 100 \times \exp(=\nu m\varphi/55.51)$, where ν is the number of ions per molecule of electrolyte and m is the molality of the solution (18). Details may be found in (19). Sorption and desorption curves were obtained by sequentially exposing the same nail to increasing and decreasing RH, respectively. At each RH the samples came to constant weight in 3–5 days, which was taken as the measure of equilibrium.

DATA ANALYSIS

The relative pressure of water vapor, $x = p/p_0$, was calculated as $RH/100$. This value is essentially the water activity, a_w (20). The amount of water absorbed by the nail samples at each value of x was expressed as the adsorption volume, v, calculated as (g of water/g of dry tissue). The water uptake values in the plot are the mean values of nail clipping data considered as from one donor (n = 6) and three sets of intact cadaver nails (n = 4–5/donor). These variables were related according to equilibrium sorption isotherms used previously to describe water uptake in other keratinized tissues. In particular we have considered the D'Arcy-Watt model (8), modified to exclude the linear term as described in (21). This relationship is:

$$v = \frac{Bbx}{1 + bx} + \frac{Ccx}{1 - cx} \tag{1}$$

where B, b, C, and c are disposable constants. The first term on the right hand side of equation 1 is a Langmuir-like term describing primary adsorption; in that sense B corresponds to the monolayer volume, v_m (20). The second term describes multilayer sorption as discussed in (1). We also considered the Guggenheim-Anderson-deBoer (GAB) model (equation 2):

$$\frac{v}{v_m} = \frac{cKx}{(1 - Kx)(1 - Kx + cKx)} \tag{2}$$

Equation 2 is a modified BET isotherm (20); indeed it was called just that in a previous report from our group (21). The parameters c and v_m are the binding constant and the monolayer volume, respectively. The parameter K [equal to $1/a_0$ in (21)] is a positive constant, with a value less than 1, that is associated with weakly bound water in the second and successive layers surrounding the keratin fibers. The GAB model has found wide applicability in hydrophilic polymer (22,23) and food (24) systems and has considerable theoretical justification (25). In particular, non-unit values of K arise from a phenomenon known as "jamming" that is physically more realistic than the unimpeded sorption process inherent in the BET model (25).

The above sorption models were fit to individual data sets (nail clippings and intact cadaver nails) for both the sorption and desorption phase, using nonlinear regression (SigmaPlot®, Jandel Scientific). Values were compared by one-way ANOVA, and differences having $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The results of human nail water sorption studies conducted in our laboratory are shown in Figure 1, along with data from Baden (17). The results show that the human nail has a maximum water uptake of ~0.3 g H_2O/g dry tissue at 100% RH. The magnitude of water sorption in nail clippings was comparable to that of intact cadaver nails, but showed less hysteresis, as discussed below.

The nail water sorption isotherm is a Type II isotherm with a characteristic hysteresis between uptake and desorption, similar to that observed in wool, hair, and porcupine quills (8). The hysteresis may be attributed to an unfolding of the keratin bundles on adsorption that is not immediately reversed on desorption (5). The hysteresis was more pronounced in some nail samples than in others. In particular, it was higher in intact cadaver nails than in nail clippings. We postulate the observed difference may be related to the differences in surface-to-volume ratio. The hydrostatic pressure exerted by the adsorbed water on the samples having a higher surface-to-volume ratio (clippings) would be less in comparison to those having a lower ratio (intact nails). Other explanations are possible. El-Shimi and Princen (26) suggested that the hysteresis effect for human stratum corneum was correlated with the process of aging and the associated change in stratum corneum elasticity. Watt (8) argued that hysteresis in wool was associated with relaxation processes taking place within the fibers and also discussed the influence of the drying process. All of these arguments invoke the concept of stress within the microfibril

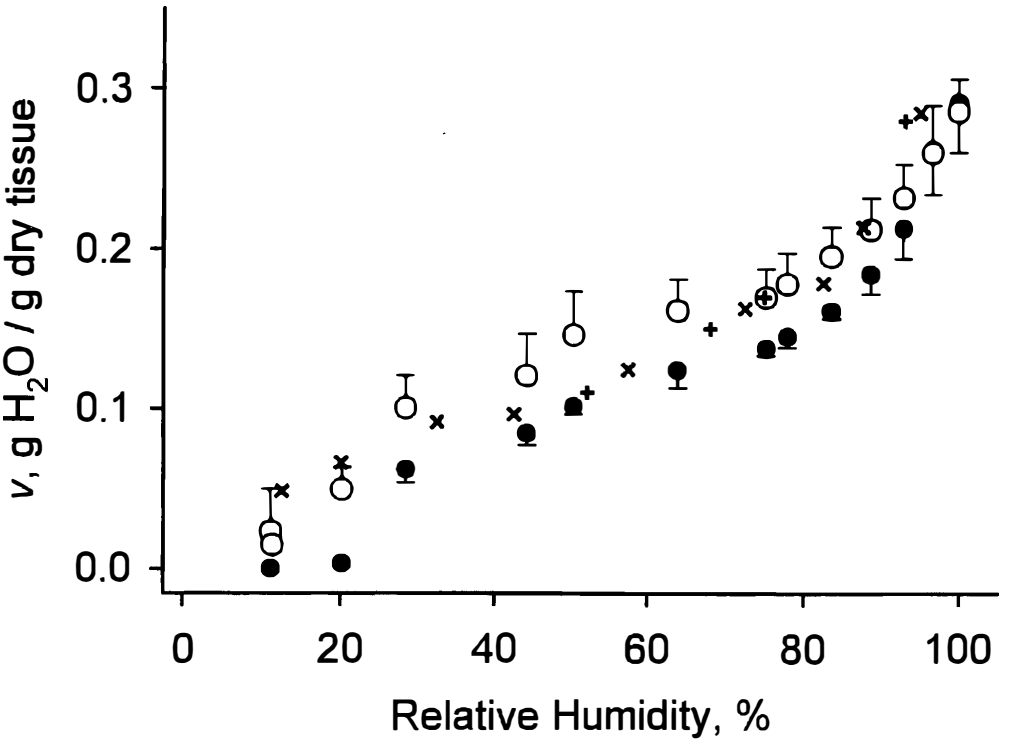

Figure 1. Equilibrium water sorption for human nail (mean ± SD of four donors, n = 4–6/donor). Uptake (●), desorption (○). Data from Baden (17) (×) and from Turek *et al.* (28) (+).

network as the tissue swells and deswells. The freezing of the cadaver nails during storage may have been a factor; however, it should be noted that the water in nail is highly bound to keratin. Based on an analogy with stratum corneum at comparable water contents, it is likely there is no freezable water in cadaver nails (27).

Compared to other reports of nail water sorption (17,28), the water uptake values obtained in our laboratory are slightly lower throughout the entire range of relative humidity. They are also somewhat more scattered. The lower values may be related to temperature; the literature values were obtained at 25°C, whereas our studies were performed at 32°C. Similar results have been seen in wool–water vapor isotherms where the amount of water adsorbed at any specified humidity decreases as the temperature is increased (8). Such a dependence is expected for an exothermic sorption process (29). The scatter may be related to the use of nonsaturated salt solutions for most of the equilibrations. While the RH of these solutions can be accurately calculated (18), it can drift as the solution exchanges water with the environment. Saturated solutions provide a more reliable RH—see Yabuza (30) for an excellent discussion.

Figure 2 shows a comparison of water uptake in nail to that for other keratinized tissues, i.e., the hard keratins horn (9), wool (31), and hair (31), and the soft keratin found in stratum corneum (21). There is a major difference in the total water uptake between soft and hard keratins. This may be attributed to the presence of high levels of cystine

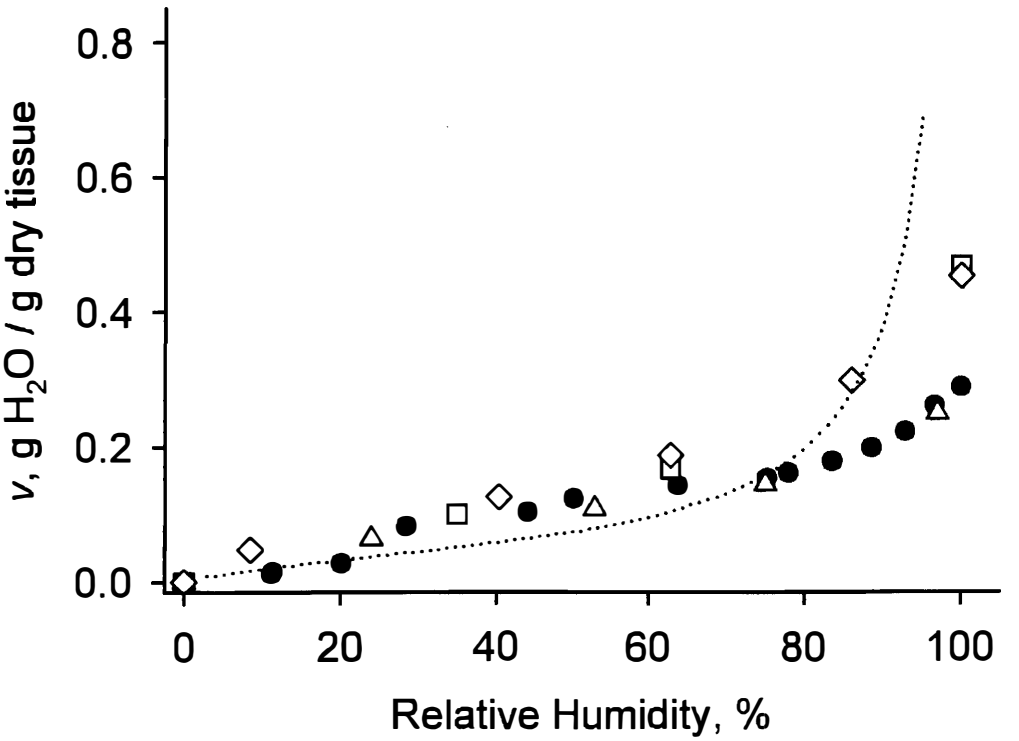

Figure 2. Mean equilibrium water sorption for human nail (●), horn (9) (△), hair (31) (◇), wool (31) (□), and human stratum corneum (21) (dotted line). The nail data are the mean of the uptake and desorption values in Figure 1.

in hard keratin, which restricts excessive swelling due to the disulphide linkages. It is also evident that the shape and the magnitude of the nail isotherm are closest to that of horn. In the range of 30–60% RH, nail, horn, wool, and hair sorbed approximately the same amount of water. Phylogenetically, these hard keratin structures arise in tissues that provide a static function and are exposed to more pressure and friction than other keratinous membranes; hence they require a fair amount of rigidity. Excessive water uptake would soften these structures, detracting from their function. Their low water-holding capacity corresponds to this requirement.

Fits of the D'Arcy-Watt and GAB models to the uptake and desorption data are shown in Figure 3, and the regression parameters are shown in Tables I and II. Both models fit the entire data set with $r^2 > 0.99$. The GAB model fit the data in the region of higher water activity very closely but missed the initial bend of the isotherm at water activity in the range of 0.3–0.5. At lower RH (>25%), water molecules are principally bonded to hydrophilic sites [primarily amino and carbonyl sites (8)] by hydrogen bonds; this region may be described by a Langmuir isotherm. The values of the monolayer volume, v_m, estimated from the GAB model, were 0.06 and 0.11 g H_2O/g dry tissue for the sorption and desorption phases, respectively. The desorption value was higher than that for uptake, possibly due to the larger number of water-binding sites available on desorption. At higher humidity, additional water is adsorbed, leading to multilayer sorption, with the tissue being saturated with 0.3 g H_2O/g dry tissue at 100% RH.

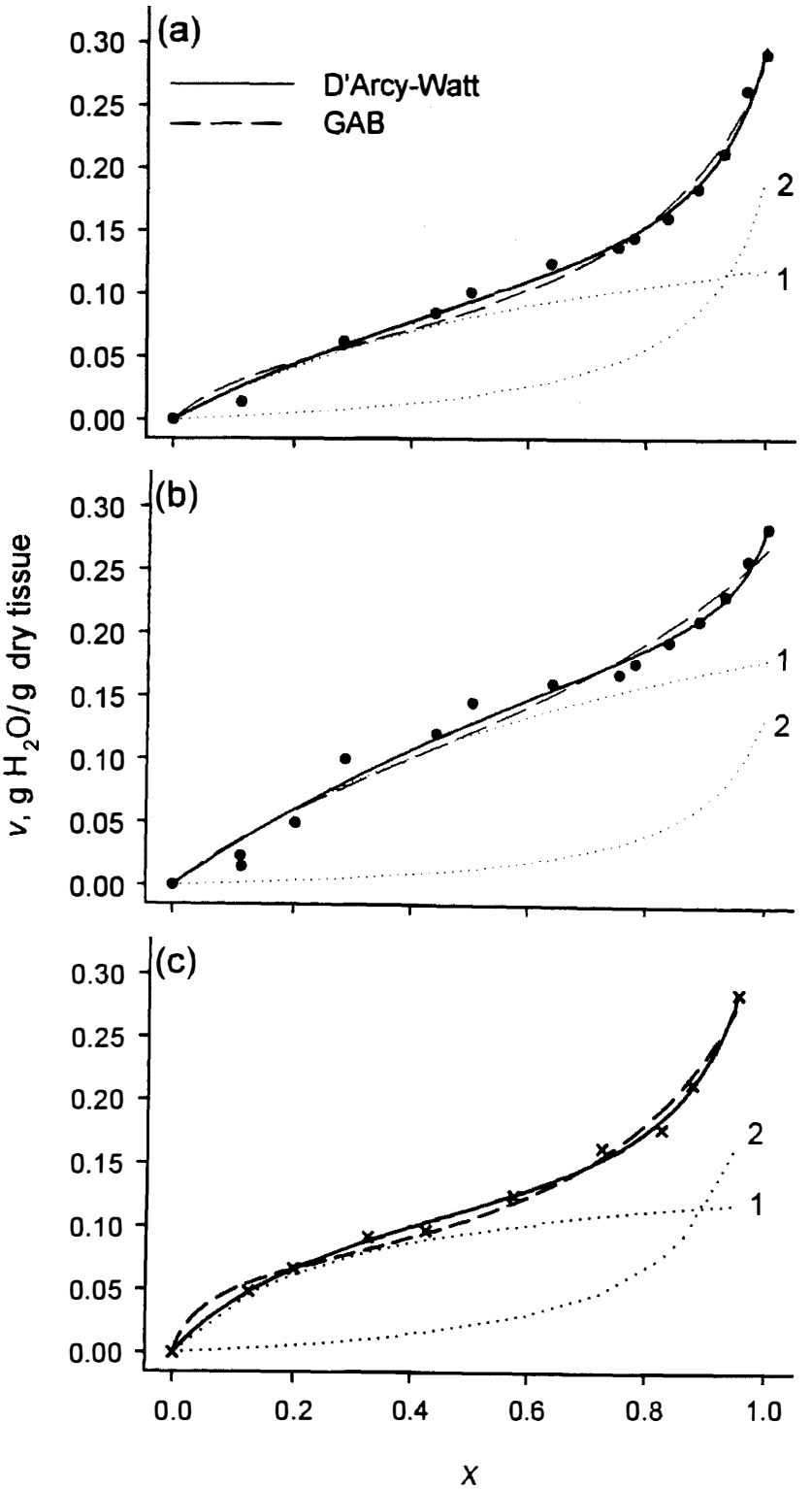

Figure 3. Isotherm model fits to the data in Figure 1. The solid lines represent the D'Arcy-Watt model and the dashed lines represent the GAB model. The dotted lines are components of the D'Arcy-Watt model as discussed in the text. Uptake (a), desorption (b), and data from Baden (17) (c).

Table I
Regression Parameters from Least Squares Fits of the D'Arcy-Watt Model to the Data in Figure 1

Parameters	Units	Uptake (Figure 3a)	Desorption (Figure 3b)	Baden (17) (Figure 3c)
b	—	1.21 ± 0.53	0.98 ± 0.31	3.48
B	g H_2O/g dry tissue	0.22 ± 0.07	0.37 ± 0.08	0.15
c	—	0.89 ± 0.01	0.89 ± 0.08	0.92
C	g H_2O/g dry tissue	0.022 ± 0.007	0.017 ± 0.019	0.024
n	—	12	14	9
r^2	—	0.9980 ± 0.0009	0.9963 ± 0.0018	0.9991
s	g H_2O/g dry tissue	0.0085 ± 0.0024	0.0121 ± 0.0035	0.0057

Table II
Regression Parameters from Least Squares Fits of the GAB Model to the Data in Figure 1

Parameters	Units	Uptake (Figure 3a)	Desorption (Figure 3b)	Baden (17) (Figure 3c)
v_m	g H_2O/g dry tissue	0.061 ± 0.009	0.112 ± 0.021	0.068
c	—	8.68 ± 1.22	6.41 ± 1.22	24.5
K	—	0.79 ± 0.08	0.62 ± 0.11	0.80
n	—	12	14	9
r^2	—	0.9965 ± 0.0012	0.9935 ± 0.0033	0.9974
s	g H_2O/g dry tissue	0.0108 ± 0.0002	0.0150 ± 0.0033	0.0092

The water sorption data for human nail was described by the D'Arcy-Watt model with an average r^2 of 0.9980 for sorption and 0.9963 for desorption (Table I). Examination of the model components (dotted curves in Figure 3) shows that most of the water sorbed at up to 80% RH (x = 0.80) may be described as strongly bound water according to this analysis. This component, which corresponds to the first term on the right-hand side of equation 1, is shown by the curves labeled "1." Water associated with multilayer formation (the second term in equation 1) is labeled "2." This observation is consistent with findings of Wessel *et al.* (32), who, using Raman spectroscopy, showed that mainly bound water is present in human nail. In a similar vein, El-Shimi and Princen (26) argued from a D'Arcy-Watt analysis that multilayer formation is a minor component of the overall sorption isotherm for wool and hair, but a significant component for human stratum corneum. The difference was attributed to the difference in the total equilibrium water uptake of these tissues.

The parameters B and C may be thought of as the number of strong binding sites and the number of water clusters, respectively (33). In comparison with Baden's data, our value of B for uptake was directionally higher and that of C was directionally lower, implying a higher ratio of bound water to multilayer water. This difference could be related to temperature, as Baden's work was conducted at 25°C and ours at 32°C. In other systems analyzed by this procedure, including wool (34) and plant seeds (33), the values of both B and C have been found to decrease with increasing temperature. The dependence for C is not surprising (higher temperature => less water clusters); however, the dependence for B is not anticipated from the theory. By analogy with the BET model, where the binding parameter varies as $\exp[-(Q_l-Q_v)/RT]$ (20), the constant b

describing the interaction between sorbate and sorbent at the strong binding sites should be more clearly temperature-dependent. The threefold lower value of b for our data vs Baden's (which arises from lower water sorption in the range of x = 0.1–0.2) may reflect this temperature dependence.

Our analyses do not imply that adsorbed water in nail may be strictly classified as "bound" and "free." A range of energy states for adsorbed water molecules is highly probable, and multilayer water is not the equivalent of bulk water (8,25). The analysis is consistent with findings for nail (32) and other hard keratins (26) that most of the adsorbed water in these tissues is strongly bound to protein fibers and that the contribution made by multilayer formation is small.

CONCLUSION

Human nail is saturated with ~30% water at 100% RH and 32°C and shows a characteristic hysteresis between uptake and desorption. Of the several models tested, the sorption isotherm is best described by the D'Arcy-Watt model.

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