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Rheology of stratum corneum—I: A molecular interpretation of the stress-strain curve

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Synopsis—Some of the problems involved in obtaining mechanico-chemical data on STRATUM CORNEUM are outlined. A conditioning process is suggested which allows reasonably consistent ELASTIC MODULUS data to be obtained for this complex substrate.

The effect of RELATIVE HUMIDITY, over the range 30–100%, on the elastic modulus of stratum corneum is dramatic, the value changing from 2×10^9 N m^{-2} at 30% relative humidity to 3×10^6 N m^{-2} at 100% relative humidity. An attempt has been made to interpret these changes in modulus at a molecular level by comparing data from other keratinous substrates and using POLYMERS as models.

INTRODUCTION

During normal body movements, certain areas of the skin are subjected to relatively large deformations, and, in this connection, the importance of the intrinsic rheological properties of the material have been recognized by a number of investigators. Medical studies have centred on the mechanical properties of the whole skin (1, 2) but the region of interest to the cosmetic chemist does not extend much below the skin's surface (3–5). Lesions arising from the effects of soaps and detergents on the skin are probably confined to its outer layers. Large extensions which are rapidly reversible can be imparted to healthy skin without any detectable damage. However, over a long period damage does occur, one manifestation of the

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ageing process being loose, flaccid skin which undoubtedly has lost its power of elastic recovery.

The stratum corneum being the outermost layer of the skin, is the first part of the body to be subjected to environmental insult. It has two prime functions; to regulate water loss and in consequence body heat, and to act as a barrier against the penetration of foreign substances. Corneum disruption will result in these functions being impaired to a greater or lesser degree. There is evidence to suggest that the physical integrity of the stratum corneum depends upon effective plasticization of the material (6) and, since water is probably the most efficient plasticizer, upon its water content. The effect of water as a plasticizer should be indicated by a change in the rheological properties of the corneum with its water content.

Owing to difficulties involved in obtaining accurate dimensions, particularly thickness, for isolated stratum corneum, no previous attempt has been made to quantify rheological data, and as a result comparisons between different types of corneum and other related substrates such as hair and wool have been impossible. This communication attempts to introduce a quantitative element into the study of the mechanical properties of stratum corneum. Load/extension curves have been obtained for narrow strips of pigs' ear stratum corneum and elastic (Young's) moduli have been calculated in the rh range 30–100%. Although some attempt has been made to interpret these data at a molecular level the main purpose of this initial paper is the establishment of techniques.

Experimental

Stratum corneum was removed from the skin of pigs' ears [in both structure and function there is little difference between animal and human stratum corneum (7)] by trypsin digestion of the underlying tissues (8). Excised skin was incubated at 37° overnight with the dermis side in contact with a solution of trypsin (1%) and urea (2 M), the pH of the solution having been adjusted to 7.2 with sodium bicarbonate. After such treatment the stratum corneum could be teased away from the underlying tissue in a water bath. The corneum was then removed from the bath and dried in the form of a flat sheet. Trypsin was obtained from BDH (reagent grade, activity not less than 0.5 Anson units g^{-1}) and urea and sodium bicarbonate were of *Analar* grade (BDH).

Rheological tests were carried out on narrow strips of stratum corneum (approx. 0.2×10^{-2} m wide) which were cut with a stainless steel punch. Calculation of the desired parameter, the elastic modulus, required a know-

ledge of the thickness of the stratum corneum. Microscopic measurement of frozen transverse sections from several pigs' ears gave an average value of the thickness of 15×10^{-6} m and this has been used throughout in the modulus calculations. Even along a single section the variation in thickness values obtained was extremely large, ranging from $8–25 \times 10^{-6}$ m and as it was not known whether this represented the true variation in corneum thickness or was simply due to poor sectioning, an average value was undoubtedly the best estimate. Direct measurement of the thickness of excised corneum by means of a micrometer gauge could not be made due to inhomogeneities in the form of small hairs, patches of lower epidermis (Malpighian layer) which adhered tightly to the corneum undersurface, and the variously wrinkled nature of the dried corneum sheet.

An *Instron* tensile tester fitted with the lowest load cell (the A cell) operating at high sensitivity (load range 2–40 g) was used to obtain elastic moduli. The extension rate was usually about 10% min^{-1} except for very low modulus samples which required higher rates (never greater than 50% min^{-1}). An environmental chamber in the form of a *Perspex* box surrounded the corneum sample, and *Instron* clamps and temperature and humidity within the box were controlled with a Vapor-temp apparatus (Blue M Electric Co.). Accurate measurements of temperature and relative humidity (rh) were made with a wet and dry bulb thermometer arrangement situated close to the sample within the box. Throughout this work the temperature was $25 \pm 0.5°$ and relative humidities were accurate to $\pm 2\%$. A relative humidity run involved the determination of the elastic modulus of a single corneum sample at a number of relative humidities in the range 30–100%. During a run the rh was always increased and an equilibration time of 20 min was allowed at each rh. Measurements of elastic modulus as a function of time at particular rh values indicated that 20 min was more than long enough for samples to equilibrate.

The method by which *Instron* load cells measure load involves a certain amount of yield in the interior of the cells. In the case of the A load cell, the yield (extension or deflection coefficient) is quoted as 2.54×10^{-6} mg^{-1} (*Instron, Maintenance Manual,* **1**). The extension obtained from the *Instron* chart is therefore a composite of that of the sample plus the load cell yield ($2.54 \times$ number of g load $\times 10^{-6}$ m in this case) and the appropriate subtraction must be made to find the sample extension. The nature of this correction is such that its significance depends on sample length for a material of a particular modulus. Longer samples will show greater extensions on application of the same load and a length will be reached at which the yield correction is negligible. This critical length for stratum corneum

was found to be about 4×10^{-2} m at 30% rh. Since it proved to be extremely difficult to produce corneum strips of 4×10^{-2} m or greater with any consistency, 1×10^{-2} m to 2×10^{-2} m lengths were normally employed and the appropriate yield correction applied. In fact, a more accurate yield factor 2.40×10^{-6} mg^{-1} was calculated for the particular load cell used in this study by the use of whiskers of pure metals (copper, tin, lead) for which the dimensions and elastic moduli were known accurately.

Infra-red studies were carried out using a *Perkin-Elmer 225* double beam spectrophotometer calibrated over the range 4 000–400 cm^{-1} by means of water vapour, polystyrene and CO_2. The instrument was fitted with *Perkin-Elmer* wire grid polarizers so that the dichroic ratio of any absorption band could be measured if required. The samples of stratum corneum used were well below the average thickness (<10 μm) and were examined in the transmission mode using both normal and polarized radiation.

Results

A typical load/extension curve for stratum corneum, at 30% rh, up to the break point is shown in *Fig. 1*. This includes: a shoe region probably due to the taking up of slack in the various connection points of the clamping system, a Hookean region (stress $\propto$ strain) and the beginnings of a yield region. The total extension observed was about 1%. However, due to sample non-uniformity, the break point extension was not reproducible. Elastic modulus values were obtained from the slope of the Hookean region and were given by:

$$E = \frac{F \times l}{A \times (dl - K)} \text{ N m}^{-2}$$

where F is the force in newtons, A the cross-sectional area, l and dl the initial length and extension respectively, and K is the correction for load cell yield. The initial length of the sample was the distance between the *Instron* clamps at the start of the extension run plus the distance AB in *Fig. 1* and the total Hookean extension for application of a 20 g load was represented by the distance BD. *Fig. 1* is in fact a facsimile of an *Instron* chart recording and in order to convert the chart distances into the actual increase in length of the sample it is necessary to multiply by the ratio of the cross head to chart speeds which are known.

Initially, elastic modulus values obtained for stratum corneum (50–60 samples) at 30% rh and 25° were randomly distributed in the range

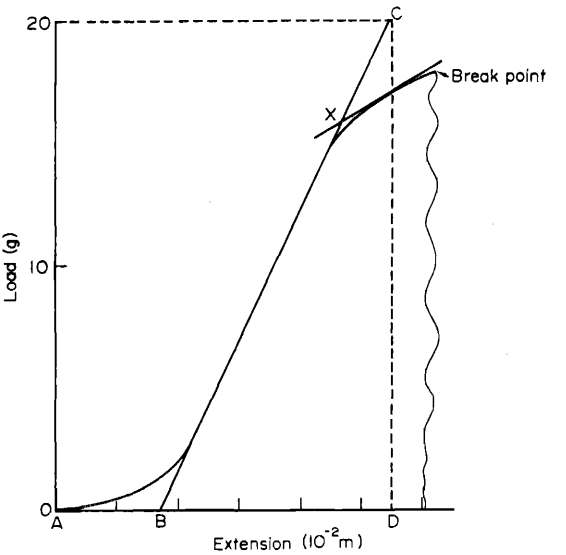

Figure 1. Typical load/extension curve for stratum corneum at 30% rh.

2×10^8 to 3×10^9 N m^{-2}. Such a wide variation seemed unacceptable in terms of dimensional inhomogeneities. The largest possible error arising from the uncertainty in sample thickness would result in a two-fold change in modulus. Another possible source of error is from the holes present in the corneum in the form of hair follicles and sweat ducts, but due to the small area occupied by such appendages any structural non-uniformity arising from this would be expected to be small. It has been mentioned that the dried corneum sheets were variously wrinkled and flatter samples tended to have higher modulus values.

In an attempt to standardize the physical state of the corneum a conditioning procedure was instituted. If corneum samples which had low modulus values ($\approx 10^8$ N m^{-2}) at 30% rh were extended a fixed percentage of their initial length at high rh and allowed to dry out under slight tension (2 g load) in their extended state, the 30% rh modulus value increased substantially. Samples which had high modulus values to begin with displayed only small increases in modulus when treated in this way. *Table I* shows the effect of various conditioning extensions on high and low modulus samples.

Very little increase in the modulus of either sample is observed for extensions greater than 10% and future samples were conditioned by this amount. After conditioning, the modulus values for all corneum samples fell in the range 1–4 $\times$ 10^9 N m^{-2}. High resolution ir spectra were recorded for stratum

Table I

Effect of mechanical conditioning on the elastic modulus of two stratum corneum samples

% conditioning extensions (N m^{-2})	0	5	10	15	20	25
$E_{Low} \times 10^{-9}$	0.2	0.8	1.5	1.7	1.8	1.7
$E_{High} \times 10^{-9}$	3.0	3.2	3.6	3.8	3.6	3.8

corneum samples both before and after conditioning and the principal amide band frequencies associated with protein in the α-helical conformation (9) (amide A = 3285 cm^{-1}; amide B = 3064 cm^{-1}; amide 1 = 1653 cm^{-1}; and amide 11 = 1543 cm^{-1}) were unchanged. This shows that no significant conformer change in the main protein component (keratin) took place due to conditioning extension. A possibility exists that the protein component of the stratum corneum orientates itself in the direction of stretch, leading to higher modulus values. Examination of the conditioned samples using polarized ir radiation showed that none of the strong band absorptions had acquired any dichroism. From these results it was concluded that the increase in modulus values observed after conditioning was due mainly to

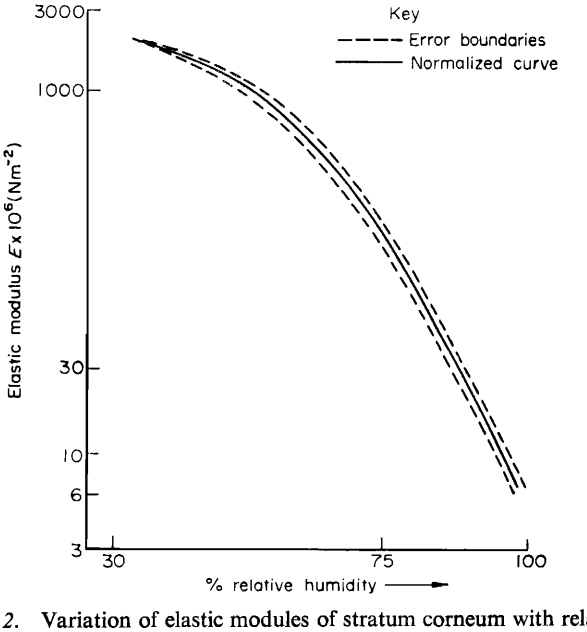

Figure 2. Variation of elastic modules of stratum corneum with relative humidity. - - -, Error boundaries; ——, normalized curve.

sample unwrinkling and that the conditioning procedure described was a valid technique for obtaining reasonably consistent modulus values. A tentative explanation of the fact that the modulus becomes constant after a conditioning extension of 10% will be given below.

Graphs of modulus versus rh were constructed for conditioned corneum strips in the range 30–100% rh. *Fig. 2* is a composite of several of such graphs in which modulus values are plotted on a log scale for clarity. To reduce the effects of sample heterogeneity further, the graphs have been normalized to the average modulus value of 2×10^9 N m^{-2} at 30% rh. All points were within experimentally acceptable distances from the average curve ($\pm 2\%$ in rh and $\pm 5\%$ in the elastic modulus).

Yield point extensions were difficult to determine with accuracy at low rh values owing to the fact that brittle fracture generally occurred. The data listed in *Table II* were taken as the point of intersection of tangents to the load extension curve before and after yield (see *Fig. 1*, point X).

Table II

Approximate yield point extensions for stratum corneum at various relative humidities

RH (%)	Extension at yield point (%)
30	1.0
50	1.5
75	2.0
85	3.5
90	8.0
100	20.0

Discussion

Before entering upon a discussion of the rheological properties of the stratum corneum it is necessary to outline briefly the salient compositional and structural details of this substrate in so far as they are known (10). Stratum corneum is a proteinaceous cellular material, the main component being a soft form of the fibrous protein keratin (60–70% of the dry weight) which is contained within the cells. A protein of different amino acid composition to that of the keratin is the principal constituent of the corneum cell membrane (11). Both of these proteins contain the cross-linking amino acid, cystine, which constitutes 2–3% of the keratin and 7–8% of the cell

wall protein. Adjacent corneum cells do not appear to be in contact at all points along their connecting surfaces and the areas where adhesion does occur have been called desmosomes (12). Other important constituents of the corneum are lipids (5) and so-called hygroscopic materials including free amino acids, simple peptides and salts of organic acids (3). The keratin is principally in the α-helical conformation (13) and bundles of these helices (protofibrils) are randomly distributed in the plane of the corneum surface within the cells. In the following discussion it is assumed that the load applied to the corneum is carried mainly by protein molecules although a role for lipids in this respect cannot be ruled out. Evidence is available (14) which suggests that the cell membrane protein is more important than the keratin in determining the rheological properties of stratum corneum but the term 'protein' when used will be understood to refer to both of these proteins. The types of bonds most likely to occur between and within protein molecules considered here are hydrogen bonds, salt linkages and disulphide bonds.

The two most studied keratinous materials in terms of their stress/strain properties are hair and wool (15, 16) and it is informative to compare these substrates with stratum corneum. The stress/strain curve for hair or wool comprises a Hookean region ($\simeq$2% extension), a yield region ($\approxeq$2–25% extension) followed by a post-yield region (25% to break). The Hookean region is believed to arise mainly from the elastic straining of intramolecular hydrogen bonds formed between peptide groups in the keratin helix. In the yield region, as the name suggests, large extensions are produced by very small increases in stress. In this region, hydrogen bonds are broken and the onset of the α–β conformational transformation can be observed (17). Larger stresses are required to extend the keratin in the post-yield region of the curve and molecular changes are thought to comprise further α–β change with some straining of the extended β chains. At low rh, fracture prevents observation of the stress/strain characteristics of stratum corneum much beyond the Hookean region. A yield region can be observed at high rh values and possibly some semblance of a post-yield region, although the transformation from yield to post-yield is not as distinct as for hair and wool.

The comparatively high modulus values observed for stratum corneum at low relative humidities (*Fig. 2* and *Table II*) suggests that a similar mechanism of extension (bond stretching) to that suggested for hair and wool is operating in this region of the curve. The variation in modulus with rh is, however, much more pronounced for corneum (about a factor of 1 000 between 30 and 100%) than for either hair or wool (about a factor of 4).

Another significant difference between these two types of 'keratinous' substrate is the variation in yield and break point although these are difficult to measure accurately. The extension at which turnover from the Hookean to the yield region occurs is sensibly constant at about 2% for animal fibres in the rh range under discussion, while for stratum corneum an increase from about 1–20% is observed in extension at yield on going from 30 to 100% rh. These observations suggest that a different molecular mechanism of extension is operating for stratum corneum at high rh values.

Water is probably the most efficient plasticizer of these three proteins and it is known that stratum corneum is capable of inbibing five or six times its dry weight of water at 100% rh (18). Hair and wool on the other hand, absorb only about 35% of their dry weights. It has been shown that the substantial water-absorbing power of stratum corneum is due to the presence of water-soluble hygroscopic materials which are trapped within the substrate by lipids (3). A possible mechanism of water absorption by corneum will be suggested in a following paper. Whatever the mechanism, there is no doubt that excessive hydration of the corneum occurs and that this is manifested in its rheological properties. The simplest model which adequately explains the change in elastic properties of stratum corneum with rh is provided by the transition of a polymer from a glassy to a rubbery state. At low rh the corneum shows the characteristics of a polymeric glass in which large chain movements are restricted and extension takes place by the stretching of bonds. In the wet state where hydrogen bonds and salt linkages are hydrated but the disulphide bonds remain intact, the corneum protein chains form a lightly cross-linked entanglement network similar to that of a rubber.

Some explanation of the lack of change in modulus with conditioning above 10% extension can now be offered. After the corneum is unwrinkled, further extension at high rh results in chain disentanglement. A stress build-up in the corneum is observed during the drying out process but this can be alleviated by slightly shortening (1–2%) the corneum strip. Upon returning to 30% rh the protein chains are frozen in a new 'configuration', and a different set of hydrogen and ionic bonds are formed. There is no reason to suppose that the numbers of such bonds will be grossly different from the number in the unwrinkled state after 10% extension, and in consequence the Hookean modulus is similar. There are two possible explanations for the absence of ir dichroism which the process of chain straightening outlined above should have produced; either the effect is too small to be observed, or the protein of the cell membrane rather than the keratin is

being extended (the ir spectrum of stratum corneum is mainly due to the keratin which is the largest component of this substrate).

In the conditioning process after the extended corneum had been dried out it showed no tendency to contract over a period of several months. This has obvious implications with regard to the wrinkling of skin with ageing. If over a period of time the water content of the corneum is reduced, the ability to return to its original configuration after extension will be less. That is to say it will gradually lose its rubber-like qualities.

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