FRACTURE TOUGHNESS DESIGN IN EQUINE HOOF WALL

BY

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ABSTRACT

This study applied an engineering fracture mechanics approach to the investigation of the fracture resistance of Equine hoof wall. The fracture mechanics parameters of stress intensity factor (K), strain energy release rate (G) and the J-integral (J) were used to determine the effect of notch orientation and specimen hydration on fracture, using the compact tension test geometry. The J-integral was found to provide the best indication of fracture behaviour because it is not based on strict linear elastic behaviour, as are K and G. Hoof wall has greatest fracture resistance for cracks running vertically, parallel to the tubular structures found in hoof wall keratin. For fully hydrated material tested in this direction, the mean critical J value at failure was $1.19 \times 10^2$ J/m$^2$. This was nearly three times larger than the value determined for the weakest orientation, in which the crack ran parallel to the material between the tubules. Hydration level was also found to profoundly affect the fracture toughness. An intermediate hydration level (75% RH) gave the highest mean critical J values ($2.28 \times 10^2$ J/m$^2$), which represented a two-fold increase over fully-hydrated and dehydrated material. These results have been related to the morphology and function of the hoof in the living animal.
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INTRODUCTION

The skeletal components of higher plants and animals function through sophisticated design tactics which exploit the mechanical properties of their structural materials (Jeronimidis, 1976). The form a structural tissue takes will result from an interaction of the requirements of function and the availability of materials with appropriate properties. The investigation of the mechanical properties of structural biomaterials and the morphological design on which those properties is based can yield important information about the type and extent of this form-function relationship in biological systems.

The ability of an organism to maintain a biologically relevant internal environment is only possible through isolation and protection from the external environment. Rather specific functions are required of the tissue which acts as this barrier. The interface material employed by vertebrates, in one organizational form or other, is the fibrous protein composite keratin, produced specifically by epidermal tissue. The epidermis of terrestrial vertebrates has several unique aspects. Unlike other body tissues, epithelia have no organized extracellular structures. Therefore, the epidermis contains no blood vessels and has no direct blood supply. All structures formed by the epidermis are cellular and gain their functional properties from their cellular contents, membrane connections
and the geometric organization of the constituent cells.

Epidermal cells synthesize a diverse group of proteins, called keratin, that are laid down within the cell. Keratin is a protein composite consisting of two phases, a fibre phase composed of long slender α-helical fibres and an amorphous protein matrix. Each fibre is composed of nine microfibrils arranged in a ring with two more possibly in the centre (Fraser et al., 1962). The individual microfibrils are likely arranged in a coiled-coil rope-like structure of α-helical protein molecules (Fraser and Macrae, 1980) with two or possibly three α-helices per microfibril (Crewther, 1976; McLachlan, 1978). The matrix of this material is composed of amorphous proteins which are crosslinked to each other and to the α-helical fibres through disulphide bonding at cystein residues (Wainwright et al., 1976). The cells responsible for keratin synthesis, termed keratinocytes, die in the final stages of their differentiation when inter- and intra-molecular disulfide cross-links are established. The extensive molecular cross-linking produces a stable composite material of long thin fibres embedded in an amorphous matrix.

Since the epidermis is composed solely of dead cellular tissue, there is no means of modifying its structure after it is laid down. Rather, it is renewed through the continual growth of the basal cell layers. In this way nutrients required for cell growth and development are secured from the underlying dermal tissue, and older external tissue is replaced as it
becomes worn and damaged. Because this type of deposition precludes remodelling or regeneration of epidermal tissues while they are in use, all mechanical requirements must be anticipated at the time of tissue deposition. Specialized cutaneous appendages adapted for specific mechanical functions have been developed where the organization and constituent proteins have been modified to match their very specific functions (Fraser and Macrae, 1980). Examples of these special appendages in vertebrates include scales, feathers, hair, beaks, and claws.

One particularly interesting cutaneous appendage is the Equine hoof. The specialized mode of locomotion in the horse (perissodactyl, ungulagrade) and the large size of this animal put extreme mechanical demands on the epidermal structure which covers and protects the distal end of the limb. We would then expect the morphological organization of the hoof wall to represent a response to the special mechanical demands of its function.

The horse hoof is a very complex structure that likely requires many mechanical properties of its tissues. The hoof wall will require a certain degree of compressional stiffness in order to support the foot and resist deformation. A lack of stiffness will result in the hoof deforming without supporting the animal's weight. Ultimate strength, the maximum force that a material can withstand before breaking, will also be required at a reasonable level in order for the hoof to bear the weight
of the animal without breaking. Thus, the hoof material must exhibit a combination of reasonable stiffness and strength.

Compressional stiffness (rigidity) in fibrous composite materials (such as keratin) results from the linking of the fibrous components together as a single bonded unit. This linkage stops the individual components from sliding relative to each other under the influence of stress produced in bending deformations (Wainwright et al, 1976). The long thin fibres are able to resist compressional forces without buckling, the normal response of long thin structures, because the matrix ties the individual fibres together into a single rigid unit (Fig. 1). The stabilization of the fibres in hoof keratin is due to extensive cross-linking of the fibre and matrix phases (Fraser and Macrae, 1980). In addition, rigid bonding at cell boundaries through specialized cell junctions stabilizes the material at the cellular level (Leach, 1980).

A composite material of this sort will, thus, be both stiff and strong. However, the rigid interconnection of components creates a continuous medium which may allow flaws to pass easily through the material from one phase of the composite to another (Gordon, 1980). Glass, for example, is both stiff and strong, but because flaws pass easily through it, glass is a poor structural material. Fracture in a rigid material occurs as a result of flaws or cracks within the material that create stress concentrations (Gordon, 1976). Loads, which would normally be evenly distributed within the
Fig. 1. Stiffness produced in a fibre reinforced composite by crosslinking the fibres. In A the fibres are able to move relative to each other allowing bending and buckling to occur. In B the matrix links the fibres into a single functional unit which will not buckle.
material are concentrated at crack tips, and can cause localized fracture. The binding of the fibres and the stiffening of the matrix allow these stress concentrations to be passed from fibre to fibre causing catastrophic failure, or fracture, of the material. Once adequate levels of stiffness and strength are achieved in hoof keratin, the most important mechanical consideration becomes the resistance to crack growth. In materials science this resistance to fracture is termed fracture toughness. It is produced through the development of stress dispersing and energy absorbing mechanisms within the material.

This study was designed to investigate the fracture of hoof wall and determine which aspects of the tissue morphology influence fracture toughness. Previous studies of hoof wall mechanics have concentrated on stiffness and strength aspects (Goodspeed et al, 1970; Dinger et al, 1973; Butler, 1976; Butler and Hintz, 1977; Leach, 1980). These studies have recognized several variables, in addition to tissue morphology, which may influence the mechanical properties. These include water content, sample location and pigmentation. In order to investigate the fracture behavior and determine the influence of these variables on the fracture toughness of this material, an engineering approach to the analysis of fracture behaviour has been employed.

The application of fracture mechanics is relatively new to biological research. However, two biomaterials, wood and
bone, have received considerable attention. Wood is used as an engineering material and, like keratin, is a cellular composite. Because wood has such importance as an engineering material, fracture studies have been many and varied (Debaise et al., 1966; Schniewind and Pozniak, 1971; Jeronimidis, 1976). Wood has a complex cellular and molecular architecture that produces a variety of subtle but effective crack resisting properties. Such things as the spiral molecular orientation of some cell walls and the general cell morphology have been implicated in energy absorption and stress reduction during fracture in wood. Fracture concepts have also been used to investigate the functional design properties of bone. Again, specific morphological features, such as osteonic organization and spiral fibre orientations, have been found to play an important role in the resistance to crack growth (Piekarski, 1970; Pope and Outwater, 1971; Simkin and Robin, 1974; Bonfield and Datta, 1976; Wright and Hayes, 1977; Alto and Pope, 1979; Wainwright et al., 1976). Beyond these two materials, however, very little is known regarding fracture resistance design in biomaterials.

One of the problems in using fracture analysis to study biomaterials is that fracture mechanics has been developed expressly for engineering applications. The materials used by engineers generally have linear stress-strain properties, but this is generally not the case with biomaterials. It is often expedient to use techniques based on linear elastic properties, even if the properties are actually non-linear. This greatly
simplifies the analysis, and, within certain limits, can yield productive information. In addition, some new techniques that account more precisely for nonlinear properties and high strain conditions are available.

Structural biomaterials present several problems for the analysis of fracture that are not generally dealt with in engineering investigations. High strain conditions are frequently encountered, and often function as energy absorbing mechanisms (Gordon, 1980). Animal tissues can be extremely complex and hierarchically organized (Katz, 1980). There are many opportunities for designing systems which do not communicate stresses and energies to a fracture site (Gordon, 1980). Horse hoof is a good example of this type of structural biomaterial, and as such, it provides an outstanding opportunity to determine the applicability of these analysis techniques to this type of tissue. The evaluation of the fracture mechanics approach to the analysis of the fracture properties of horse hoof wall was also one of the purposes of this study.

The material under study and the analysis techniques employed in this investigation are not commonly encountered in biological research. Some knowledge of the complex morphology of the tissue is crucial to the understanding of the mechanical behaviour of the tissue. For this reason, a short chapter is included reviewing the known morphology of the hoof wall. In addition, a brief explanation of the theory of fracture and of
the testing techniques used in fracture analysis is provided to prepare the reader for the study which follows. A more detailed treatment of fracture mechanics can be found in Broek, 1978, Elementary Engineering Fracture Mechanics.
This section is intended as a brief review of the morphology of the epidermal hoof wall in preparation for discussions of the mechanical consequences of that morphology to be presented in sections to follow. The exterior of the foot of the horse consists of the epidermal hoof capsule (Fig. 2). The hoof wall covers the dorsal and lateral aspects of the foot. It forms an angle to the ground when the animal is standing normally of approximately 45 to 50 degrees for the front feet and 50 to 55 degrees for the rear feet (Evans et al., 1977). The wall connects proximally with the skin at a junction termed the coronet. The interior distal portion of the wall connects to the sole epidermis at a junction referred to as the white line. This junction consists of the interdigitation of hoof wall and sole epidermal structures. Interiorly the epidermal wall connects to the dermis. The connection is accomplished by the interdigitation of many lamellae which run vertically on the interior of the epidermal wall. These primary lamellae divide into a series of secondary lamellae which connect intimately with the basal membrane of the epidermal wall and complimentary primary and secondary lamellae of the dermis (Fig. 3).

The epidermal wall is avascular and contains no nerves (Sisson and Grossman, 1953). It contains three specific layers (Stump, 1967). The stratum externum constitutes the external
Fig. 2. Sagittal section of the Equine foot taken from area indicated by dotted line in A. The relationship of the epidermal wall and other tissues of the foot is depicted. CB - Coronary border, the germinative region SS - Stratum spinosum, the keratinizing zone SM - Stratum medium, fully keratinized tissue WL - The white line, the dermal/epidermal junction S - Sole epidermis PII and PIII are the second and third phalanx of the Equine limb.
Fig. 3. Cross-section of hoof wall taken from area indicated by dotted line in Fig. 2B. D - dermis, BM - basal membrane, SL - Secondary epidermal lamellae, PL - primary epidermal lamellae, SI - Stratum internum, SM - Stratum medium, SE - Stratum externum.
covering of the hoof over most of its surface. This is a very thin coating of cells produced by the periople at the proximal margin of the hoof. This coating is carried distally with the underlying stratum medium as it grows toward the distal ground contact surface. As the cells of the periople are sloughed off, the stratum externum takes on the form of a glossy varnish, and is believed to act as a water barrier due to its very high lipid content (Leach, 1980). It is gradually worn away as the hoof grows distally and may not be present at all at the distal margin of the hoof. The stratum internum is the layer adjacent to the internal tissues of the foot. This is the layer containing the primary and secondary epidermal lamellae. It forms the solid connection between the hard external wall and the internal tissues. The stratum internum is composed of a continuous layer of living cells at the basal membrane, which is the division between the epidermal and dermal components of the integument. The stratum medium comprises the majority of the hoof wall and is the layer dealt with in the mechanical investigations of this study. The stratum medium is located between the stratum externum and the stratum internum. The thickness of this layer varies with location on the hoof; it is thickest at the toe and becomes gradually thinner toward the posterior lateral margins.

The stratum medium, as with the other layers of the hoof wall and all vertebrate epidermal tissue, has a completely cellular composition. The cellular nature and manner of growth of this tissue profoundly affects the organizational form. New
wall material is produced continuously. Therefore, the stratum medium is continuous with germinative, keratinizing and mature fully keratinized tissue. The hoof wall forms on the epidermal basal membrane located at the proximal border of the hoof wall. The germinative cells develop and replicate in this position. As new cells are formed the older ones move distally to become part of the stratum spinosum (Fig.2). In this region the cells undergo cytodifferentiation and the keratinization process begins. The fibrillar patterns are established as the cells continue to be pushed distally by newer cell formation. The cells are incorporated as part of the stratum medium of the wall when the final stages of keratinization take place. At this stage the cell dies as a result of intermolecular cross-linking of the majority of its cytoplasmic constituents. During keratinization the cells flatten slightly and, in a hard keratin such as the hoof wall, specialized inter-cellular junctions are formed. These junctions closely resemble the 'narrow junctions' found by Hashimoto (1971a) in nail cells (Matoltsy, 1975; Leach, 1980).

The hoof wall contains approximately equal portions of cellular material organized in spiral tubules and intertubular material which connects the tubules. The tubules are parallel structures that run the length of the hoof wall from the proximal growth zone to the distal contact surface. Each tubule contains a central cavity, the medulla, surrounded by highly organized cellular material, the cortex (Fig.4). The medulla can contain some amorphous material. The tubules have been
Fig. 4. Scanning electron micrograph of hoof-wall keratin prepared by tearing a wet sample, showing the organization of the tubular material. C, cortex; M, medulla. Scale bar = 40 μm.
classified into three categories based on polarized light studies. Nickel (1938) believed these polarizing patterns were caused by a system of alternating spiral sheets or laminae. Wilkens (1964) disagrees with these findings and concludes that no discrete laminae exist. The crystalline fibres within the cells do have a high degree of preferential orientation and do present an overall spiralling pattern within the tubules but Wilkens attributed the difference in polarization pattern of the various tubule 'types' to differences in angular orientations of the component cells. Due to an unfortunate error in the English translation of the summary given in the original paper, the significance of this valuable work has been largely neglected by English speaking investigators in this field. In describing the organization of the tubules Wilkens used the analogy of a pine cone, saying the cells of the cortex adjoin the medulla at an angle just as the imbricated scales of a pine cone adjoin the core. The translated summary, however, replaced the term 'pine cone' with 'pin cushion' and all meaning was lost. The slope of the connection between the cortex cells and the medulla is derived from the slope of the dermal papilla on which the tubule is formed. The larger innermost tubules are formed on larger dermal papillae. These papillae are the same length as the narrower papillae, and thus, have a sharper slope in the germinative region. Sections of these tubules cut the component cells at a different angle than cells of the narrower tubules and give a slightly different polarized light pattern.
The cells of the intertubular material are similar in general shape to those of the tubule cortex, but their orientation is quite different. These cells are produced on the plane of the basal membrane, and they maintain this planar orientation throughout the hoof structure. The angle formed by the surface of these cells and the tubule axis can vary. This variation is suspected to be related to the location of inter-tubular cell formation, but the exact circumstances are not known. The long axes of these cells lie generally in the plane of the basal layer and at right angles to tubule direction. In the exterior portion of the stratum medium the intertubular fibre orientation tends to be in a more circumferential direction, while the inner tissue appears to have a more interwoven orientation. These cells are generated on the basal membrane between the dermal papillae and move distally as they are replaced at the generative surface.

The hoof wall presents a complex hierarchy of structural levels. The understanding of the integration of these levels in either a morphological or functional sense demands that the scale of each be appreciated. The keratin composite within the cells is composed of proteinacious polymers. The fibres of this composite have a diameter of approximately 7.0 nm. (Fraser and Macrae, 1980), and are themselves constructed from smaller microfibrillar units. The cells, that are filled with this fibre and matrix composite, are pancake shaped and are approximately 40-50 μm in diameter and generally 5 μm thick. At the next level of the hierarchical organization, the tubules are
seen to be generally circular or slightly elliptical in cross-section with a diameter of approximately 50 - 150 μm depending on the location in the wall. The smaller and more elliptical tubules are found toward the exterior surface of the stratum medium, while the largest tubules are found in the interior. The diameter of a given tubule is maintained over the entire length of the hoof wall. Adjacent tubules can have very different tubule diameters, however. The intertubular material lies between the tubules, and this determines their spacing.

The size of the hoof wall varies with the size of the foot and breed of horse. A normal hoof can be expected to be 8-12 cm from the coronet proximally to the distal contact surface. The stratum medium is approximately one cm thick at the toe, narrowing to a few millimeters at the posterior quarters.
The study of the effects of flaws on the mechanical behaviour of materials is termed fracture mechanics. The response of a material to the physical circumstances created by a flaw is a property of that material, as are such properties as stiffness and strength. However, the analysis of the mechanical response to a flaw is a complicated issue. This section is intended as a brief introduction to the concepts and theories underlying the study of fracture in structural materials. It will also include a synopsis of the analysis techniques employed in this study and some of the practical limitations inherent in the application of these theory based procedures to the case of 'real' materials.

The process of crack propagation depends upon two criteria: (i) the presence of adequate stress conditions at the tip of a crack or flaw, and (ii) the availability of sufficient energy to drive the crack extension (Broek, 1978). The pattern of stress within an object under conditions of load will be profoundly affected by the presence of a flaw (Griffith, 1921). The energy state of the object will also be affected by the interaction of strain energy within the body of the object resulting from the load and the energy cost of crack growth. Neither of these two conditions alone is sufficient to cause crack growth (Broek, 1978). In order to understand the process of fracture it is necessary to investigate the action of stresses and energies within an object under load.
An even load on a homogeneous object will produce a uniform internal stress pattern, where stress is defined as the force per unit area over which the force acts. If a flaw is present in the object, no stress will be carried by the flawed portion of the object, and the stress that would have passed through the region the flaw occupies is forced to divert around the flaw (Fig.5). This causes a concentration of stress at the tip of the flaw. The magnitude of absolute stress present in this situation can be much greater than in the rest of the object (Fig.6). The high stress state caused by the flaw will produce a localized strain (length of deformation per unit length) in the bonds connecting the remaining material at the tip of the crack (Fig.7). If the stress at the crack tip reaches a critical value it will begin to rupture the molecular bonds, and the material will begin to fracture. The surfaces of the crack cannot carry any stress. Therefore they act as stress boundaries and dominate the distribution pattern of the stress concentration at the crack tip. The crack geometry and loading mode can be used to determine the stress pattern in a test situation (Irwin, 1958). The loading forces will only affect the intensity of the stress field and not influence its distribution pattern. The stress related fracture properties of a material can then be characterized by an analysis of the stress intensity field under the conditions that cause failure of the material. Irwin (1958) determined that a characteristic term, the stress intensity factor (K), existed for the specific spatial distribution of stresses caused by a flaw. The stress
Fig. 5. The effect of a notch or flaw on the path of stress in a loaded object.

Fig. 6. The magnitude of stress levels across an object containing flaws or notches (after Currey, 1962).

\[ \sigma \] - level of stress
Fig. 7. Stress at a crack tip causing molecular bond strain and rupture.

Fig. 8 The Griffith criterion of fracture. Energy is absorbed during fracture through such processes as bond strain and breakage. The energy is directly proportional to the length of crack. The strain energy released by fracture is also a function of crack length. Catastrophic fracture occurs when the slope of strain energy release is of equal magnitude to the slope of energy absorbed.
intensity factor could then be determined for the critical circumstances which would cause failure of a material.

An alternative method of viewing fracture depends upon the energy balance that exists within a material under stress. A load applied to an object will be resisted by the straining of molecular bonds. The extension of the molecular bonds constitutes work (or energy) performed on the system. The inter-molecular work developed in this manner can be stored elastically, and is referred to as strain energy. As a crack grows, the volume of material which carries no load increases, and as a consequence strain energy will be released from the adjoining material which had resisted stress before the crack passed. For geometrical reasons the strain energy released will be a function of the length of the flaw, while the energy necessary to rupture the connecting bonds at the tip of the flaw (the fracture process) will be a function of the area of new surface created. These two energy terms will then interact as a function of crack growth (Fig.8), and the flaw will begin to grow catastrophically when an incremental growth results in the release of more stored strain energy than is absorbed by the creation of the new crack surface (molecular bond rupturing) (Griffith, 1921). The energy related fracture properties can then be characterized by determining the rate of the strain energy release (∂U/∂a = G) (Griffith, 1921).

Provided the material under question exhibits linear elastic properties, the two parameters, stress intensity factor
(K) and strain energy release rate (G), will describe the resistance to cracking, or 'toughness', a material possesses. However, real materials do not possess ideal elastic properties. Non-elastic deformation at the tip of a flaw can alter the stress distribution field and absorb strain energy. If the extent of plastic deformation is small compared to the stress fields involved, the stress intensity factor and strain energy release rate can still adequately describe the conditions responsible for fracture; if not, other fracture parameters must be employed to analyse the fracture process.

The limit of the applicability of K and G to a fracture analysis situation is dependent on the size of plastic deformation at the crack tip. Because the material in the stress concentration zone adjacent to the crack tip is constrained by less stressed material farther from the stress concentration, stresses are produced across the thickness direction of the test specimen (Pook and Smith, 1979). This situation is termed plane strain. As long as it is maintained, the analysis techniques can predict the stress and energy states at the crack tip, and the elastic fracture criteria apply. If plastic deformation occurs and the extent of the plastic zone approaches the thickness of the sample, the stress relieving deformation will reach the specimen boundaries and the thickness stresses will be reduced. This will result in a plane stress situation arising in the region assumed to be under plane strain. Under these circumstances the stresses at the crack tip are not described by linear elastic mechanics,
and the stress intensity factor and strain energy release rate
do not apply.

The analysis of fracture under greater degrees of plastic
deformation prior to failure is an area of much recent
investigation in materials science, and an alternative analysis
parameter, the J integral (Rice, 1968), has been proposed for
the evaluation of fracture under these conditions. The J
integral is a path independent evaluation of the
elastic-plastic energy field at the flaw tip and represents the
potential energy available for crack propagation (Landes and
Begley, 1972). As such, it is a generalized relation for the
energy release due to crack propagation (Broek, 1978). It
applies in spite of appreciable plastic deformation at the
crack tip. Due to the path independent aspect of the
evaluation, the most convenient integration contour can be
selected, generally the specimen boundaries. In this way, all
energy available and required for crack growth can be
determined. For fully elastic materials the value of J is
equivalent to the value of G, since the potential (total)
energy (J) is only in the form of elastic energy (G). This
fracture parameter also depends on plane strain conditions
predominating in the region of the crack tip.
MATERIALS AND METHODS

I. Acquisition and Preparation of Samples.

Whole horse hooves were obtained from a commercial abbatoir within two days of the animal's slaughter. While at the abbatoir the hooves were kept cold but not frozen. Thirty apparently normal hooves were taken from 12 horses. The hooves were classified by pigmentation at this stage. Three classifications were used: (1) obviously darkly pigmented, (2) obviously unpigmented, and (3) moderately pigmented. These three classes were quite distinct. Vertical slices of the mid-toe region (Fig. 9) of the hoof were cut with an industrial band saw and the dermis was removed with a scalpel. The cleaned strips of hoof epidermis were then labelled, placed in double plastic bags to maintain their natural moisture content and refrigerated. A hacksaw was used to cut the strips of hoof wall into approximately 1.2 cm blocks. The blocks were then shaped to appropriate length, \( W \), and height, \( D \), dimensions with a flat file. Care was taken to ensure the shaping was done accurately and all sides of the samples were parallel. However, because some of the cutting was done by hand, variations in absolute dimensions were unavoidable. These variations were accurately measured and accounted for in the analysis. The samples were washed with distilled water throughout the cutting process to ensure that frictional heat would be kept to a minimum. The cutting and
Fig. 9. The location of hoof wall material tested. The most distal and proximal portions were removed. The centre portion of the wall was cut sagittally producing strips of hoof wall of approximately 1.7 cm. width. These were then cut into five blocks which were later cut into the Compact Tension test geometry. T - top, UC - upper centre, C - centre, LC - lower centre, B - bottom.
shaping procedure allowed the individual samples to be cut appropriately to the major morphological feature, that of the tubule axis. The outer surface of the specimen blocks were filed parallel to the tubule axis and perpendicular to all other sides. The outer face of the finished sample block was then affixed to a small plexiglass mount with polymeric adhesive (Superglue). These were then labelled, placed over distilled water in a small vacuum chamber and refrigerated.

It was considered critical to the analysis procedure that the lateral sides of the test specimen be absolutely parallel. To ensure this an Isomet variable speed metalurgical sample saw was used to cut individual test samples from the shaped blocks. The samples were cut at a slow rate while being continuously washed with distilled water to eliminate heating. After sawing, the test pieces were individually placed in sealed 2 dram vials and labelled. These were then refrigerated. Holes for the clevis and load pin attachment were drilled with a Maxitmat 7 drill press using a 1.8 mm. drill bit.

Notches were cut in these test specimens using a single edge razor blade held in a jig attached to the drill press. The test piece was set vertically and clamped in a vice attached to the milling slide carriage. The drill press head was then lowered onto the test piece using the vertical leadscrew. The single edge razor blade gave a narrow, sharp notch.

II. Testing Procedures.
The loading system and specimens were attached firmly inside specially designed sealed constant environment test chambers that allowed samples to be hydrated and tested while in a controlled environment. Full hydration was produced either by placing the test samples over distilled water in the test chambers or by covering the samples with distilled water while in the test chamber. Covering the samples with water made no difference to the final water content of the samples (mean water content was 38.4% by weight for samples hydrated in the vapor phase; while the mean for samples covered by water was 39.6%), but reduced the hydration time by a factor of two.

The dehydrated condition was produced by preparing the test samples wet and then placing them in the environment test chamber over phosphorus pentoxide drying agent (BDH Laboratories). Periods of 2 to 3 weeks in this situation produced specimens which had an apparent water content of 5% by weight, as indicated by weight loss after oven-drying. It is assumed that a period of this length in 0% RH would remove all water from this material. The loss of weight observed after oven-drying probably represents a loss of some volatile aspect of the tissue itself. Intermediate hydration conditions of 53% RH and 75% RH were produced by placing saturated solutions of Mg(NO₃)_2·6H₂O and NaCl in the environment test chambers (Meites, 1963). These relative humidity levels produced water content values of 11.7% by weight at 53% RH and 18.1% by weight at 75% RH.
In metallurgical investigations compact tension (CT) specimens are generally fitted with clip-type displacement gauges which measure displacement of the sample directly. In this case the small size of the samples and the environment chamber test regime did not allow this procedure. In this study displacement was measured as test machine cross-head displacement. Since the cross-head was securely connected to a clevis and pin arrangement, it was considered to be an accurate estimate of the load pin displacement. All tests were conducted on an Instron testing machine Model 1122 fitted with a proportionally driven chart recorder. The chart recorder was run at a proportional drive of 20:1 for the 100% and 75% RH samples and 50:1 for the other less compliant hydrations. The cross-head rate was arbitrarily standardized at 5 mm/min. This rate was chosen simply because it was slow enough to give dependable responses from the testing apparatus. Force was recorded with a 500 Kg max. load force transducer. Maximum loads for these samples approached 120 N (12.25 Kg).

The test procedures were adapted from the American Society for Testing and Materials (ASTM) Standard Test Procedure E-399-81 "Plain-strain Fracture Toughness of Metallic Materials Employing the Compact Tension Test Geometry". Due to constraints on the amount of material available from the horse hoof, the specimen sizes used were smaller than those recommended by this procedure. However, the relative dimensions recommended were maintained (Fig.10). The compact tension test allows controlled conditions to be produced in reasonably small
Fig. 10 The Compact Tension fracture test specimen geometry. $W_T$ - total width of sample, $W_1$ - width of sample between loading point and notched edge, $W$ - width of mechanically effective sample, $A_T$ - total length of notch, $A$ - mechanically effective notch length, $B$ - specimen width.

Fig. 11 Specimen loading system used in Compact Tension fracture tests. Upper load rod was grasped by clamping system of testing machine. Base load rod ran horizontally and was firmly attached to environment test chamber.
scale specimens. The limitations of the application of these procedures are discussed later. The load fixtures used consisted of the clevis and load pin arrangement advised by the prescribed procedures (Fig. 11).

During a test the clevis load rod of the sample was attached to an aluminium adaptor in order to allow attachment of the pneumatic grip with which the testing machine was equipped. The clevis load rod was secured with two set screws, and the head of the adaptor was clamped by the pneumatic grips. The compliance of the system was tested by using an unnotched piece of aluminium of similar size to the test samples in place of the hoof specimen. The unnotched aluminium sample was expected to be much stiffer than any test specimen; therefore, the compliance in this test would indicate the compliance of the system during the testing of the hoof samples. The test system deflected a maximum of $1.3 \times 10^{-6}$ m/N. In the experiments run at full hydration the system compliance accounted for less than 4% of the specimen compliance and has been ignored. In experiments at reduced hydrations, where sample stiffness increases, the relative system compliance was larger. In the 0% RH samples the system compliance was as much as 25% of the sample compliance. This error in deflection measurement was consistent and was accounted for in the calculations for tests run at all hydration levels except 100% RH.

During the course of a test only a single seal cap was removed from the test chamber to allow access to the load rod.
This kept to a minimum the exchange of environmental humidity. The testing of all 12 samples in a test chamber lasted a maximum of two hours. Immediately upon completion of these tests the samples were individually removed, weighed accurately (Mettler H31 balance, 0.0001 g) and measured for the dimensions of thickness B, total width \( W_t \), distance from the edge of the sample to the centre of the load pin hole \( W_i \), height D, total original notch length \( A_t \) as well as fracture angle.

The dimensional lengths of B, \( W_t \) and D were measured using a Helios micrometer (.01 mm). The notch length, \( A_t \), was measured under a binocular dissecting microscope using a pair of sharpened screw-type protractors as fine calipers. The depth of the load pin hole, \( W_i \), was measured in the same manner. \( W_i \) was measured for both sides of the sample, and the mean was taken as the measured value. The ligament length, \( W \), was determined by subtracting \( W_i \) from the total specimen width \( W_t \) . The original notch length \( A \) was determined by subtracting \( W_i \) from \( A_t \) . Fracture angle and intertubular cell orientation were measured on a Wild binocular dissecting microscope fitted with a rotating stage. This allowed accurate determination of all angles involved. When the crack varied in direction, the original direction was taken as the fracture angle.

Hydration of the individual samples was taken as the wet weight, measured immediately after the test, minus the dry weight, measured after at least three days in an oven at
approximately 80°C. The temperature of the desication was maintained at this level to ensure the morphology would be left intact for subsequent histological studies. Further drying beyond the three days produced no change in weight.

III. Analysis of the Fracture Data.

The mechanical test records were digitized by measuring force at standardized intervals of load pin displacement. Intervals of $2 \times 10^{-4}$ m. were used for the 100% and 75% RH samples while intervals of $4 \times 10^{-5}$ m. were used for the lower hydration states. The stress intensity factor ($K$) was calculated from the equation:

$$K = \left( \frac{P}{B W^\gamma} \right) f(a/W),$$

where:

$$f(a/W) = \frac{(2+a/W)(0.87 + 4.6a/W - 13.3a^2/W^2 + 14.7a^3/W^3 - 5.6a^4/W^4)}{(1 - a/W)^{3/2}}$$

In these equations $P$ is the load, $B$ is the specimen thickness, $W$ is the specimen width and $a$ is the apparent crack length. The strain energy release rate ($G$) was calculated using the compliance calibration method: where $C = \text{the compliance calibration relationship.}$

$$G = \frac{p^2}{2B^2} \frac{\partial C}{\partial a}$$

The specimen width term was squared in this analysis because the compliance calibration curve had been normalized to the specimen width (i.e. $C = q/P \times B$) (from Broek, 1978). The determination of the
J integral required that the energy put into the specimen during a test be measured. This was done by integrating the force deflection record at the same deflection intervals as the force measurements. Cuttings of the appropriate segments from photocopied test records were accurately weighed. A standard area was weighed from each test record in order to calibrate the individual record weight to energy relationship. The J integral value was determined from $J = -1/B(\partial U/\partial a)$ (Rice, 1968).

In order to define a critical failure point for the test specimens, the maximum sustainable load ($P_{\text{max}}$) was determined as the load value of the test record given by the intersection of a 5% deviation from the initial compliance (Fig. 12), according to the recommended procedure (ASTM—E399). $P_{\text{max}}$ was used rather than $P_{\text{max}}$ to determine the critical limits of all the fracture parameters analysed in this study. The validity test of $P_{\text{max}}/P_{\text{q}} < 1.10$, originally designed to verify the stress intensity factor (ASTM—E399), was used to eliminate test records from the data set which did not apply to the analysis techniques employed in this study.

IV. Compliance Calibration.

Composite materials pose a formidable problem for the accurate determination of the actual crack length during fracture (Gaggar and Broutman, 1975). The use of a hydration chamber test situation also restricted access to the test sample during testing. For these reasons a compliance method of
Fig. 12. Analysis of Compact Tension test record.

- $C_o$: initial compliance determined from slope of linear portion of record
- $P_m$: maximum load
- $P_q$: failure point defined as the load achieved at the intersection of a line with 5% lower slope than the initial linear portion of the record.
- $U$: the energy absorbed by the specimen over a given displacement $q_1$ to $q_2$. This was empirically measured from the test record.

\[
e = 0.05 \lambda
\]
\[
\frac{P_m}{P_q} \leq 1.10
\]
\[
U = \int_{q_1}^{q_2} f(q) \, dq
\]
crack length determination was employed. This technique has been successfully used in studies of synthetic composites (Gaggar and Broutman, 1975) and in the study of bone (Wright and Hayes, 1976).

The compliance technique uses the elastic response characteristics of test specimens calibrated over a range of crack lengths. The procedure involves the determination of the elastic spring constant (reciprocal of the load displacement curve) for various notch length (A) to specimen width (W) ratios. For comparisons of different materials and specimen geometries the compliance can be normalized for both modulus and specimen width. In this study compliance curves were determined for each hydration condition. Therefore all samples used in the calibration had similar moduli and a normalization for this factor was unnecessary. The samples were normalized for the variations in specimen width. The values of the width normalized elastic spring constant were then calculated as \((q/P) \times B\) for as many extensions as possible until a decrease in the slope was seen or until five values were obtained. These values were then averaged to determine the initial compliance for the sample. This was then plotted against the measured initial notch length to specimen width ratio \((A/W)\) to yield the compliance calibration curve. Since specimen compliance is a function of \(A/W\), the instantaneous crack length \('a'\) to specimen width ratio \(a/W\) could then be determined for any point in the test from the measured compliance taken from the continuous mechanical test record.
It is important to recognize that the \( a/W \) value determined in this manner represents only the apparent crack length to specimen width ratio. The compliance due to any plastic deformation field at the crack tip, as well as any microfracture damage zone will be included in the \( a/W \) calculation. This method also treats all cracks as though they were progressing parallel to the original notch. This was not always the case in this study, however. The fracture analysis techniques used in this study characterize the initiation of fracture and cannot be applied to the large scale, continuous crack growth. The crack growth at the critical point of failure was found to be small enough in all samples for any deviation to be considered negligible.

V. Tensile Modulus and Yield Strength Measurement.

Tensile tests of thin uniform samples were conducted to determine Young’s modulus (E) and yield stress. Young’s modulus was measured in order to determine the presence and/or the degree of any anisotropy in this material. The yield stress was determined in order to estimate the extent of plastic zone formation in the compact tension test pieces.

Tensile test pieces were cut in the same manner as the CT samples. The use of the Isomet metallurgical sample saw again ensured that the sides of the samples were parallel. Test specimens were cut from the mid-toe region and only those samples coming from above the vertical centre region (5.5 cm from the corium) were used. Both tapered and untapered samples
were used, but it was found that untapered samples gave reliable results while being far easier to cut. Therefore, they were more generally used. Sample dimensions varied but each sample was accurately measured prior to testing. Samples were in the order of $6 \times 10^{-4}$ m thick, $4 \times 10^{-4}$ m wide and $2.5 \times 10^{-3}$ m long. The samples were clamped above and below using pneumatic sample grips, leaving $1.2 \times 10^{-3}$ m between (original length of test sample).

Due to specific stress patterns produced by this type of clamping procedure, the displacement produced in this tests cannot accurately be determined from cross-head movement alone. The strain in the test pieces used in this study was, therefore, measured with a video dimension analyser (VDA). The change in distance between two surface marks on the test sample, placed well away from the sample clamps, was used to determine sample displacement, while the force was determined from the 500 Kg force transducer of the Instron testing machine. These were recorded together on an Esterline-Angus X-Y plotter, which gave a continuous record of total force and displacement.

VI. Histology.

Selected fracture test samples were prepared for scanning electron microscopy. After dehydration to determine hydration state, the test sample was glued to an EM stub using silver conducting paint. These samples were then coated with a thin layer of gold, using a Mikros vacuum evaporator (model VE10).
These prepared samples were then viewed in a Cambridge Stereoscan 250 scanning electron microscope. Photographs were taken with a Poloroid 545 camera and Poloroid P/N film.

Thick sectioning (5-10 microns) of wet tissue was accomplished using a steel knife on an American Optical Co. '820' microtome. The density and consistency of the hoof material made embedding conveniently unnecessary. Light micrographs were taken using either a Wild polarizing compound microscope fitted with a Wild camera or a Wild dissecting microscope fitted with an Asahi SP500 camera. All light micrographs were taken using Kodak Technical Pan film.
RESULTS

Two types of mechanical tests were conducted in this investigation. Initially, tensile extension tests were used to determine the general stress-strain properties of hoof wall keratin and to investigate the effect of specimen orientation and hydration on these properties. Following these initial studies, an extensive analysis of fracture properties was carried out to evaluate the fracture toughness of hoof wall keratin and to determine the effect of pigmentation, orientation and hydration on toughness. This analysis employed the compact tension test procedure.

I. Stress-Strain Properties: Tensile Tests.

The tensile modulus and the strength of hoof wall keratin varies widely with hydration. Therefore, this account will begin with a description of the properties of the fully hydrated tissue and then compare these to properties shown for other hydration conditions. Typical stress-strain curves for fully hydrated material are shown in Fig.13. The initial portion of the stress-strain curve has a low slope due to slack in the loading system. Once the specimen is stressed directly a linear stress-strain relationship is seen, which can be used to determine a Young's modulus (E) for the material. Beyond the initial linear elastic region the material yields and the slope
Fig. 13. Typical stress-strain curves from tensile tests of fully hydrated hoof wall keratin.
- Stress direction parallel to the tubules
- Stress direction perpendicular to tubules
E - Young's modulus. Samples failed at the specimen grips.
$\sigma_{5\%}$ - stress level achieved at the point intersected by a line with a 5% lower slope than the initial modulus.
decreases. The yield point, defined as the point on the stress-strain curve intersected by a line having a slope of 5% less than the slope of the initial linear region, occurred in this case at a strain of about 0.02. In the post-yield region the material was able to go through a great deal of strain (91% of total strain) with a modest increase in stress (44% of maximum measured stress). The samples generally failed at the test grips, and consequently the true, ultimate strength and extensibility of the material could not be measured for most samples.

The yield in the fully hydrated state was considered to indicate a change in the behaviour of the material. The stress-strain curve resembles that shown by amorphous polymeric materials in their glass transition. Indeed, it is believed that the matrix polypeptide chains of hard keratin composites, such as hoof wall, are convoluted but relatively inflexible (Fraser and Macrae, 1980). It is likely that the yield stress indicates the stress level at which the weaker, secondary bonding forces are disrupted within the matrix. The post-yield region, which allows a great deal of strain with a small increase in stress, would indicate an extension of the convoluted matrix polymers. The high proportion of crystalline fibres and the relatively high crosslink density within the matrix presumably limits the extension in the post-yield region to about 20-30%. For comparison, unfilled, lightly crosslinked rubbers can be extended as much as 600%.
A. Orientation Effects.

Table 1 summarises the information concerning tensile tests performed on this material. Since the hoof tubules constitute a consistent morphological feature, they were used to designate the orientation of the test sample. Samples in which the tubule axis was oriented parallel to the loading direction were called longitudinal tests while samples in which the tubule axis was perpendicular to the loading direction were termed lateral tests. The longitudinal samples correspond to the vertical aspect of the hoof wall and indicate the properties in that direction. The lateral samples correspond to the lateral aspect of the wall and indicate the properties in the direction parallel to the ground contact surface.

As shown in Fig.13, the tensile properties of this material appear reasonably similar in these two orientations. The data in Table 1 show that at the maximum hydration level there are no significant differences in stiffness or yield stress. Though not significantly different at this hydration level, the modulus and yield stress of the lateral samples did appear marginally greater than the longitudinal samples. The modulus of a fibre composite will depend upon the relative angle of the individual fibres to the applied stress, and on the proportion of fibres with a component of their orientation aligned with the direction of the stress (Wainwright et al, 1976). Although the obvious parallel arrangement of the tubules
Table 1. Tensile Modulus of Hoof Wall Keratin.

E - Young's Modulus, S.E. - Standard Error, n - sample size, RH - Relative Humidity level.

<table>
<thead>
<tr>
<th>RH</th>
<th>Orientation</th>
<th>E (x10^9 N/m^2)</th>
<th>S.E.</th>
<th>n</th>
<th>Yield Stress (x10^7 N/m^2)</th>
<th>S.E.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>Long.</td>
<td>.410</td>
<td>.032</td>
<td>19</td>
<td>.918</td>
<td>.042</td>
<td>19</td>
</tr>
<tr>
<td>100%</td>
<td>Lat.</td>
<td>.485</td>
<td>.035</td>
<td>24</td>
<td>1.18</td>
<td>.036</td>
<td>22</td>
</tr>
<tr>
<td>75%</td>
<td>Long.</td>
<td>2.63</td>
<td>.362</td>
<td>5</td>
<td>3.89</td>
<td>----</td>
<td>1</td>
</tr>
<tr>
<td>75%</td>
<td>Lat.</td>
<td>2.30</td>
<td>.222</td>
<td>14</td>
<td>3.14</td>
<td>.470</td>
<td>5</td>
</tr>
<tr>
<td>53%</td>
<td>Long.</td>
<td>3.36</td>
<td>.629</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53%</td>
<td>Lat.</td>
<td>5.32</td>
<td>1.07</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>Long.</td>
<td>14.6</td>
<td>.071</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>Lat.</td>
<td>5.66</td>
<td>1.23</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Long. - Tubule axis parallel to applied stress
Lat. - Tubule axis normal to applied stress
might suggest that the hoof wall is therefore strongly reinforced in the longitudinal direction, polarized light sections of the hoof wall show a marked lateral orientation of the fibres within the intertubular component. Because the tubular and intertubular components are present in roughly equal quantities, the resulting mix of fibre reinforcement yields a material with nearly equal properties in both directions. In fact, Leach (1980) found the compressional modulus in the lateral direction to be 1.48 times that of the longitudinal orientation. Thus, it appears that the laterally oriented intertubular material is an important, and possibly dominant, mechanical feature of hoof wall keratin, even though the longitudinally oriented tubules provide a dominant structural feature.

B. Hydration Effects.

As seen in Table 1, the tensile modulus of hoof wall keratin is highly dependent upon the hydration state of the test sample. Hoof wall shows more than a twenty fold increase in modulus between 100% and 0% RH in longitudinal tension. The intermediate hydrations show a progressive increase in modulus as the hydration is decreased, in both the lateral and longitudinal orientations. No significant difference is found between the moduli of the two orientations except at absolute dehydration. The low modulus determined for the lateral orientation in the fully dehydrated condition may be an
artifact of the testing specimen size. At very low hydrations the material becomes extremely stiff and brittle. Major discontinuities within the structure of the material will act as stress concentration sites in a material with these properties (Currey, 1962). In the tensile samples the tubule diameter was approximately 1/3 to 1/2 the specimen width. Thus, the tubules could cause serious stress concentrations in the laterally oriented samples, and these stress concentrations might lower the apparent modulus of the material. Alternatively, it is possible that hydration affects the properties of the intertubular material differently than the tubular material or that the variation in orientational architecture causes the apparent difference at the lowest hydration. Leach (1980) has suggested that these orientations of hoof wall keratin under compression respond differently to dehydration. Unfortunately, there is not enough evidence provided in this study to make any conclusions on this point.

A comparison of the stress-strain behaviour of representative longitudinally oriented tensile samples is given in Fig. 14. Noting that energy is equivalent to force times displacement, the area under the stress-strain curve represents the energy absorbed by the material under the loading process. The long extensions which occur after the yield point allow a great deal of energy to be absorbed by the 100% RH specimen. In contrast, the dryer material (0% and 53% RH) fails at higher stresses but much lower strains, making the total energy absorbed less. These changes in behaviour presumably result
Fig. 14. Representative stress-strain curves for tensile samples stressed parallel to the tubule axis at four hydration levels. Arrowheads indicate samples which failed at the specimen grips. The X indicates failure within the VDA monitoring range, therefore ultimate strength of material. The dotted line indicates the test record from a 75% RH sample which failed at the specimen grips.
from the increase in the secondary bonding forces that crosslink the matrix phase. Increased crosslinking will allow stress to be transmitted more directly from one reinforcing fibre to the next, and this could cause the composite to become more brittle. The lowest hydration conditions indicate the extreme of this situation where the material becomes very brittle and only small amounts of energy can be absorbed before an existing flaw spreads across the specimen. It is important to note that the 75% RH samples achieved much higher stresses than the fully hydrated samples, but many were still able to maintain the stress for long extensions. This resulted in the 75% RH samples absorbing the greatest amount of energy. All of the samples tested at 75% RH that failed between the monitoring lines of the video dimension analysing system (VDA) (ie. gave a true strength measure) gave a stress-strain curve resembling that in Fig. 14. The tensile properties displayed by the hoof wall at 75% RH suggest that under this condition the failure resistance properties of the material might be optimized. At this hydration level the ability to resist stress is combined with the ability to absorb energy through plastic deformation.

II. Fracture Investigations: Compact Tension Tests.

A. Fracture Tests at 100% Relative Humidity.

1. Compliance calibration. An integral part of the analysis of fracture with compact tension (CT) samples is the
measurement of crack length. The crack length profoundly influences the stress intensity factor (K), the strain energy release rate (G) and the J integral (J). A complex hierarchically organized composite, such as hoof keratin, poses difficult problems for visual measurement of crack growth. The crack growth in this type of material will likely be neither coplanar nor colinear with the original notch (Gaggar and Broutman, 1975), and a surface view of the crack may not represent the conditions responsible for the behaviour of the test specimen. Therefore, an indirect method of characterizing the damage due to fracture was necessary in order to analyse the fracture properties of this material. The compliance calibration method was employed, following similar techniques used in the study of fracture of other composites including bone. The compliance values were determined from CT tests, as illustrated in Fig.15. This figure shows a typical test record from a 100% RH sample. The inverse of the initial slope, normalized for specimen width, gives the initial compliance for the sample. It is assumed that no crack growth occurred in this region, an assumption that is supported by the fact that the stress-strain curve is linear. Specimens with various original notch lengths were tested, and a consistent relationship was found between the precut notch length and initial mechanical compliance (Fig.16). Polynomial regression of this data gave an empirical equation (R =0.88) which allowed the determination of the crack length directly from the mechanical test record. The crack length determined from the mechanical compliance
Fig. 15. Trace from a Compact Tension test record showing method of determining apparent crack length ($a/W$) from test record.
Fig. 16. Compliance calibration data used to determine apparent crack length. The regression was initially determined with compliance dependent on crack length ratio ($R^2 = 0.88$). The apparent crack length ratio during a test was determined from the measured mechanical compliance applied to the regression of $A/W$ dependent on Compliance.

- Mean value and standard deviation of orientation 3.
- Mean value and standard deviation of orientation 4.
- Mean value and standard deviation of orientation 2.
(apparent crack length) was calculated by taking the slope from the beginning of the test to any point on the test record during the test (Fig.15, broken line). This slope was then compared to the compliance calibration curve to determine the apparent crack length.

The compliance calibration curve at this hydration was determined using 62 samples, all with the precut notch parallel to the tubule axis. This calibration curve was used for the other three orientations as well. The initial compliance of these samples was found to fit the parallel notch calibration curve (Fig.16, specifically marked samples). Considering the similarity in tensile properties seen between orientations, the consistency in the compliance relationship at this hydration is not surprising.

2. Determination of fracture parameters. The calculation of the stress intensity factor \((K)\) and the strain energy release rate \((G)\) are straightforward and require simply the dimensions of the sample, including crack length, and force/deflection data. Determination of the \(J\) integral was slightly more complicated. The energy absorbed \((U)\) during a test was measured and plotted against the ratio of apparent crack length to specimen length \((A/W)\) for each sample (Fig.17). Polynomial regression analysis \((P<0.02\) for all regressions) gave an empirical equation for energy and crack length for a specified extension, and the partial derivative of this equation \((\partial U/\partial a)\), could be used to determine the value of \(J\) at
Fig. 17. The value of $J$ was determined from the partial derivative of energy ($U$) by apparent crack length ($a$) for each extension ($q$). The solid line represents the regression of the data. The dotted line is a visual representation of $J$. 

$$J = -\frac{1}{B} x \frac{\partial U}{\partial A} \bigg|_q$$
any point during the test.

The fracture analysis considered five independent variables which were believed to have potential effects on the fracture behaviour of hoof wall. They were pigmentation, vertical location on the hoof wall, individual animal from which the sample was taken, sample orientation and hydration state. Five dependent variables were derived from the mechanical test data: cross-head deflection (q), apparent crack growth (Δa), stress intensity factor (K), strain energy release rate (G) and the J integral (J). In order to reduce the number of interacting independent variables the data were separated into subgroups which shared as many of the independent variables as possible. A one-way analysis of variance was then conducted on the dependent variables for each of the remaining independent variables. Those independent variables which did not have significant effects on the fracture properties of this material were sequentially eliminated from the analysis. This procedure also identified independent variables which did have a significant effect and which required special consideration in further analysis.

a. Pigmentation, individuals and location. The results of the analysis of variance and multiple comparisons for the samples tested at 100% RH with similar notch orientation are given in Table 2. There were no significant differences found between the properties of the three pigmentation groups at this hydration. In the 100% RH material the level of pigmentation
Table 2. A. Results of analysis of variance and multiple comparisons for data subdivided according to pigment, individual animal and location. Location labelled as in Fig. 9, n - sample size, q - critical extension, \( a \) - critical crack length, K - critical Stress Intensity Factor, G - critical Strain Energy Release Rate, J - critical J Integral. Significance determined by Tukey's Honestly Significant Difference test \( p < 0.05 \).

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Individual</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5#9</td>
</tr>
</tbody>
</table>

\[
\begin{array}{cccccc}
q & \Delta a & K & G & J \\
\hline
\end{array}
\]

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Individual</th>
<th>Location</th>
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<td></td>
<td></td>
<td>5#9</td>
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</table>

B. Fracture test results subdivided according to location. Orientation 1, Hydration 100% RH. S.E. - Standard Error.

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>q (S.E.)</th>
<th>( \Delta a ) (S.E.)</th>
<th>K (S.E.)</th>
<th>G (S.E.)</th>
<th>J (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \times 10^{-3} )m</td>
<td>( \times 10^{-4} )m</td>
<td>( \times 10^6 )N/m^3/2</td>
<td>( \times 10^4 )N/m</td>
<td>( \times 10^4 )J/m^2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>1.4 (.06)</td>
<td>5.02 (.91)</td>
<td>1.58 (.84)</td>
<td>5.83 (.91)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>1.4 (.08)</td>
<td>4.73 (.36)</td>
<td>1.74 (.84)</td>
<td>7.42 (.76)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7</td>
<td>1.7 (.16)</td>
<td>5.74 (.41)</td>
<td>1.86 (1.54)</td>
<td>7.49 (1.04)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>10</td>
<td>1.4 (.06)</td>
<td>4.67 (.26)</td>
<td>1.72 (.69)</td>
<td>6.87 (.56)</td>
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<td>5</td>
<td>1.1 (.17)</td>
<td>5.05 (.76)</td>
<td>1.20 (1.4)</td>
<td>2.70 (.56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
was associated with different individual horses, consequently, no significant difference was found between individuals in this portion of the study either.

Having determined that there are no significant differences due to pigmentation and individual, it was possible to analyse the entire data set (tests at 100% RH and parallel notch orientation) against location from which the test samples were cut. As mentioned in the materials and methods section, there were five possible locations from which the samples could be cut. Multiple comparisons using Tukey's Honestly Significant Difference test determined that the means of several of the analytic parameters used were significantly different for the most distal portion of the hoof wall (Fig. 18 and Table 2). It is apparent from this evidence that fracture toughness decreases sharply near the distal contact surface. The material near this surface is both the oldest in the hoof wall and, consequently, the most used. It is possible, therefore, that the decrease in fracture toughness observed indicates the presence of natural flaws caused by continuous mechanical use of the material.

b. The effects of sample orientation. Since location 9 (the distal samples) was found to be significantly different in properties from the remaining samples, it was not included in the orientation analysis. Analysis of the remaining samples at 100% RH indicated significant differences in fracture characteristics related to orientation of the precut notch. The
Fig. 18. The mean critical values of stress intensity factor, strain energy release rate and J integral plotted against the vertical location on the hoof wall the sample was taken from. 1 - Top, 3 - Upper centre, 5 - Centre, 7 - Lower centre, 9 - Bottom. a - Proximal segment discarded, b - distal segment discarded.

- Stress intensity factor, K.
- Strain energy release rate, G.
- J integral, J.

Error bars indicate 95% confidence limits.
relationship between notch direction and morphological features of the hoof wall for the four orientations used in this study is given in Fig. 19. Orientation 1 consisted of samples in which the precut notch was placed parallel to the tubule axis and, therefore, the notch would run in a vertical direction in the hoof wall. Orientation 2 consisted of those samples in which the notch was placed normal to the tubule axis and, consequently, roughly parallel to the ground contact surface of the hoof. As mentioned in the description of the morphology of the hoof wall, the intertubular material orientation can vary relative to the tubule orientation. In determining the intertubular material angle for orientations 1 and 2 in Fig. 19, the mean angle of intertubular material for those samples was used. All of the samples used for orientations 3 and 4 were specifically selected so that the intertubular material was oriented at 45° relative to the tubules. The CT test specimens of these samples were then shaped in relation to the intertubular material orientation rather than the tubules. For orientation 3 the precut notch was placed normal to the direction of the intertubular material and for orientation 4 it was parallel to the intertubular material. In this way two sets of samples could be produced, each with the tubules oriented at 45° to the notch but having the intertubular material either aligned with or opposed to the advance of the crack during the fracture test.

The mean values for the stress intensity factor K were not significantly different at the 0.05 level between the four
Fig. 19. A diagram of the relationship between the precut notch and the tubular and intertubular material for four sample orientations. Note that 1 and 2 or 3 and 4 could be cut from the same sample by altering the location of the notch. Tubular material is designated by solid lines, intertubular by dashed lines.
Table 3. A. Results of analysis of variance and multiple comparisons for four orientations tested at 100% RH. Orientation labels given in Fig. 19, n - sample size, S.E. - Standard Error, q - critical extension, a - critical crack length, K - critical Stress Intensity Factor, G - critical Strain Energy Release Rate, J - critical J Integral.

<table>
<thead>
<tr>
<th>q</th>
<th>Δa</th>
<th>K</th>
<th>G</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*3&amp;2</td>
<td>1*2&amp;4</td>
<td>----</td>
<td>1*2&amp;4</td>
<td>4*163</td>
</tr>
</tbody>
</table>

B. Fracture test results according to notch orientation.

<table>
<thead>
<tr>
<th>Orientation n</th>
<th>q (S.E.)</th>
<th>Δa (S.E.)</th>
<th>K (S.E.)</th>
<th>G (S.E.)</th>
<th>J (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(x10⁻³ m)</td>
<td>(x10⁻⁴ m)</td>
<td>(x10⁶ N/m³/²)</td>
<td>(x10⁴ N/m)</td>
<td>(x10⁴ J/m²)</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>1.46 (.05)</td>
<td>4.97 (.20)</td>
<td>1.74 (.05)</td>
<td>7.09 (.40)</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>1.80 (.15)</td>
<td>6.6 (.58)</td>
<td>1.78 (.15)</td>
<td>4.71 (.66)</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>1.77 (.07)</td>
<td>5.54 (.51)</td>
<td>1.73 (.06)</td>
<td>5.92 (.39)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1.51 (.06)</td>
<td>8.08 (.99)</td>
<td>1.44 (.03)</td>
<td>3.79 (.36)</td>
</tr>
</tbody>
</table>
Fig. 20. The mean critical values of stress intensity factor, strain energy release rate and the $J$ integral for four orientations of precut notch.
- Stress intensity factor
- Strain energy release rate
- $J$ integral

Lower axis - angle between tubule axis and notch
Upper axis - angle between intertubular material and notch

Error bars indicate 95% confidence limits.
orientations analysed (Table 3). The 95% confidence limits between orientations 1 and 4 did not overlap, however (Fig. 20). This might indicate that a difference did exist but the rigorous statistical analysis test used in this study did not find it due to the small sample size available. The mean value for the strain energy release rate $G$ from orientation 1 was found to be significantly different from the values for both orientations 2 and 4 (Table 3). Orientation 3 could not be distinguished from any of the other orientations at the 0.05 level. The mean value for the $J$ integral at orientation 4 was found to be significantly different from both orientations 1 and 3 (Table 3). For this fracture parameter orientation 2 could not be distinguished from any of the others.

The lowest fracture values for these parameters are consistently associated with orientations in which the intertubular material is aligned with the precut notch (Fig. 20). At the same time, there seems to be no obvious relationship between the orientation of the tubules and the notch direction. This trend is evident in a general way for all the parameters used. The $J$ integral analysis was able to demonstrate this trend more clearly. It should be noted that the $J$ integral analysis technique allows for some plastic deformation in the material, and therefore probably provides the best method for characterizing the fracture properties of materials like hoof keratin at 100% RH. Orientations 3 and 4 had similar tubular orientations but opposite intertubular directions. The significant three-fold difference in fracture
resistance properties between these two orientations, as determined by the J integral analysis, indicates that the intertubular material plays an important role in influencing the fracture of hoof wall. A similar comparison of tubule orientation yields no significant difference in measured properties. In fact, one might anticipate that the fracture resistance would be greater for a notch orientation which ran perpendicular to the tubules (orientation 2) because the fibre orientation within the tubules would present an obstacle to an advancing crack. This is clearly not the case. This therefore provides additional evidence that the fracture is most strongly influenced by the orientation of the intertubular material. These fracture studies indicate that the hoof wall material has one orientation which is more susceptible to fracture and possesses considerable fracture resistance in any other direction. Since this single weaker direction coincides with the orientation found in the intertubular material, it must be concluded that the intertubular material plays a dominant role in determining the fracture of this material.

Photographs of the fracture path observed in the samples tested confirm this conclusion. Cracks resulting from fracture followed the direction of the intertubular orientation regardless of the tubular orientation. This effect can be most easily seen by comparing photographs of the intertubular normal (orientation 3) and intertubular parallel (orientation 4) orientations. In these two cases the tubular material lies at a consistent $45^\circ$ to the notch plane. However, the crack can be
seen to follow the intertubular orientation in spite of the fact that the CT test regime is designed to drive the crack parallel to the original notch plane (Fig. 21). This result can be anticipated since the data shown in Fig. 20 indicates that it is easier in terms of either stress or energy to fracture along the plane of the intertubular material.

Micrographs of thin sections taken from the CT samples indicate more clearly how the crack was diverted by the orientation of the intertubular material (Fig. 22). In spite of the notch being placed within a tubule, it was diverted to the intertubular material. It is also possible to see a number of fracture cracks emanating from the single precut notch. This illustrates one of the possible mechanisms available in the design of this fibre composite which increases the toughness of the material. More energy is absorbed through the creation of many surfaces than through the creation of a single fracture surface.

B. Effects of Hydration State.

Compliance calibration curves were used to determine the apparent crack length of specimens tested at other hydration levels, in the same manner as the 100% RH data. The compliance calibration relationships of these hydrations were found to be best represented by linear regressions over the notch lengths used in this study. The four calibration curves are shown in Fig. 23. All regressions were found to be significant.
Fig. 21. Photographic comparison of the crack path in samples tested in orientations 3 and 4. The white strip continuing from the end of the precut notch is the crack path. Note in orientation 3 that the crack proceeds at right angles to the axis of the notch.
Fig. 22. Polarized light micrograph of a section taken from a Compact Tension sample, showing the crack pattern that developed. The sample was hydrated at 100% RH.

N - tip of precut notch, I - intertubular material, T - tubular material, A - crack running along the intertubular-tubular interface, B - crack diverted to the orientation of the intertubular material, after passing around tubule. Scale bar = 100 µm.
Fig. 23. Compliance calibration curves determined for four hydration levels.

100% RH: \( A/W = 0.21196119 + 3.4139645 \times 10^6 (C_0) - 6.6528977 \times 10^{12} (C_0)^2 + 4.118235 \times 10^{18} (C_0)^3 \) (\( p < 0.001 \))

75% RH: \( A/W = 0.23706 + 7.18884 \times 10^6 (C_0) \) (\( p < 0.025 \))

53% RH: \( A/W = 0.257563 + 1.20914 \times 10^7 (C_0) \) (\( p < 0.025 \))

0% RH: \( A/W = 0.282584 + 1.32607 \times 10^7 (C_0) \) (\( p < 0.010 \))
1. Pigmentation, individual and location. Table 4 summarizes the multiple comparisons for pigmentation, between individuals and for vertical location on the hoof wall. As with the 100% RH samples, no difference was found in the properties associated with the three pigmentation levels except in the 53% RH samples. In this case the means for critical crack growth (A), stress intensity (K) and strain energy release rate (G) were found to be significantly different for the pigmented and partially pigmented samples. The means of the J integral for both the partial and unpigmented samples were found to be significantly different from that of the pigmented samples. This is not considered a meaningful evaluation, however. The fully pigmented samples of this hydration level were made up of samples taken from two different individuals. The analysis of variance for individuals at 53% and 0% RH revealed that the individuals concerned had significantly different properties (Table 4). Not enough data are available to determine if variation of properties exist between these individuals, and therefore, the samples were considered as a single population in subsequent analyses. No significant differences were found for any of the fracture parameters as a function of location in the hoof at the lower hydration levels.

Having dealt with the above variables, the effect of hydration state on the fracture of hoof wall can be analysed. It has been determined that notch orientation can have a profound effect on fracture properties in this material. It is important, then, to isolate orientational effects from
Table 4. Results of analysis of variance and multiple comparisons for Compact Tension test specimens at the various locations tested. Location denoted by number corresponding to Fig. 9. Orientation 1. q - critical extension, a - critical crack length, K - Stress Intensity Factor, G - Strain Energy Release Rate, J - critical J Integral. Individual animals given specific numbers, pigment classes; 1 - pigmented, 2 - unpig., 3 - partial.

<table>
<thead>
<tr>
<th>Hydration</th>
<th>q</th>
<th>Δa</th>
<th>K</th>
<th>G</th>
<th>J</th>
</tr>
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<tbody>
<tr>
<td>100% Pigment Individual Location</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>5*9</td>
<td>9*3&amp;5&amp;7</td>
<td>9*3&amp;5&amp;7</td>
<td>5*9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75% Pigment Individual Location</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
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<td>4*2&amp;5</td>
<td>4*3&amp;5</td>
<td>4*2&amp;3&amp;5</td>
<td></td>
</tr>
<tr>
<td>4*3</td>
<td>4*3</td>
<td>4*3</td>
<td>4*2&amp;3&amp;5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0% Pigment Individual Location</td>
<td>--</td>
<td>2*3&amp;4</td>
<td>--</td>
<td>4*2</td>
<td>2*3&amp;4</td>
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</tbody>
</table>
hydration effects. This has been accomplished by analysing hydration effects within one orientational group, those having the precut notch oriented parallel to the tubules (orientation 1). The effect of dehydration on tubule normal fracture (orientation 2) is also analysed.

Table 5 gives the data determined for the hydration effects analysed in this study for samples in orientation 1. The data are also plotted in Fig.24. The stress intensity factor ($K$) is found to increase significantly with a decrease in humidity from 100% to 75% RH. At this point it reaches a maximum which is maintained in the lower hydration states. This indicates that the stress necessary to initiate fracture in the material reaches a maximum at this moderate hydration level and is comparable in all of the lower hydration states.

The strain energy release rate ($G$) has its maximum at the 100% RH level, and the strain energy drops steadily to the 53% RH level. It drops less rapidly to the 0% RH level. The strain energy release rate can theoretically be considered in terms of energy necessary to produce new crack surface. A drop in $G$ indicates less energy is being absorbed in the crack growth process. The dryer samples were observed to possess a perceptibly smoother crack surface than the hydrated samples. The high values of $G$ at high hydration levels are probably related to the complex path the crack is forced to take during fracture.
Table 5. A. Results of analysis of variance and multiple comparisons (location 9 excluded for 100% RH analysis) subdivided according to hydration. n - sample size, S.E. - Standard Error, q - critical extension, a - critical crack length, K - critical Stress Intensity Factor, G - critical Strain Energy Release Rate, J - critical J Integral.

\[
\begin{array}{cccccc}
q & \Delta a & K & G & J \\
\hline
\text{all groups} & 4\times1&2&3 & 1\times2&3&4 & 2\times3&4 & 1\times3 \\
\text{different} & 1\times2&3&4 & 1\times2&3&4 & 2\times1&3&4 \\
\end{array}
\]

B. Fracture test results according to hydration level. Orientation 1.

<table>
<thead>
<tr>
<th>Hydration</th>
<th>n</th>
<th>q (S.E.)</th>
<th>$\Delta a$ (S.E.)</th>
<th>K (S.E.)</th>
<th>G (S.E.)</th>
<th>J (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(x$10^{-3}$ m)</td>
<td>(x$10^{-4}$ m)</td>
<td>(x$10^6$ N/m$^{3/2}$)</td>
<td>(x$10^4$ N/m)</td>
<td>(x$10^4$ J/m$^2$)</td>
</tr>
<tr>
<td>100%</td>
<td>30</td>
<td>1.46 (.05)</td>
<td>4.97 (.20)</td>
<td>1.74 (.05)</td>
<td>7.09 (.34)</td>
<td>11.93 (.90)</td>
</tr>
<tr>
<td>75%</td>
<td>16</td>
<td>1.11 (.07)</td>
<td>3.86 (.38)</td>
<td>3.82 (.21)</td>
<td>5.02 (.50)</td>
<td>22.82 (.31)</td>
</tr>
<tr>
<td>53%</td>
<td>28</td>
<td>.66 (.05)</td>
<td>3.08 (.18)</td>
<td>3.68 (.24)</td>
<td>2.24 (.27)</td>
<td>5.63 (.54)</td>
</tr>
<tr>
<td>0%</td>
<td>16</td>
<td>.44 (.04)</td>
<td>2.0 (.37)</td>
<td>3.71 (.26)</td>
<td>1.23 (.15)</td>
<td>8.73 (.97)</td>
</tr>
</tbody>
</table>
Fig. 24. Mean critical values of stress intensity factor, strain energy release rate and J integral at four hydration levels.
- Stress intensity factor, $K$.
- Strain energy release rate, $G$.
- J integral, $J$.
Error bars indicate 95% confidence limits.
In a general sense, the J integral appears to result from an interaction of K and G. At 100% RH, J has a moderate value. At 75% RH, J increases sharply, as did K, becoming just less than twice the 100% RH value. At 53% RH, the value of J falls to less than the 100% RH value while the value of K remains constant. This appears to be an exaggerated response to the drop in strain energy release rate (G). The J integral is apparently unchanged between 53% and 0% RH. The J integral is a measure of the total energy available for crack growth. There is no discrimination in the J integral analysis between the mechanism of crack growth (i.e. the stress at the crack tip) or the process of crack growth (i.e. the creation of new crack surfaces). It is not surprising, then, that the value of the J integral measured would represent an interaction of these two aspects of the fracture of the material. The value of J is not strictly an additive consequence of K and G because the units and method of determining these parameters are not directly comparable. The data presented here does, however, indicate that J represents a qualitative combination of the other two fracture parameters.

Since the techniques employed in this study allowed the continuous monitoring of apparent crack growth in the samples, it was possible to analyse the effect of hydration on the critical amount of crack growth at failure. Griffith (1920) showed that there should be a critical length of crack growth at which point the energy available to propagate the crack is equal and becomes greater than that necessary to cause fracture
(see Fracture Mechanics, sec. 3). A complex material such as hoof wall will not fit Griffith's model precisely, but the data from this study indicate a regular relationship between hydration and extent of apparent crack growth at the critical fracture point (Fig. 25). This indicates that at the lowest hydration level the critical crack growth is less than half that necessary at the highest hydration level. In other words, fracture is stable in the higher hydration states for a longer distance of crack growth. It should be noted, however, that even at the lowest hydration condition this material displays considerable fracture resistance, which controls the spread of a propagating crack. A mere 2 1/2 fold decrease in critical crack growth at fracture indicates a conservative response of crack growth properties in this material as a consequence of the 15 fold increase in stiffness which results from dehydration.

It is obvious from tensile tests that hydration state profoundly affects the mechanical properties of this material. At extremely low hydrations the material becomes very stiff, and appears to become very brittle. At extremely high hydration states the material has relatively low stiffness and low yield strength, but is capable of long extensions before failure. At intermediate hydration (75% RH) the material is stiff, strong and capable of absorbing large amounts of energy. The direct evaluation of fracture toughness using the CT test regime confirms these qualitative impressions from the tensile tests, but indicate that even while under conditions of extreme
Fig. 25. Mean apparent crack growth at failure for four hydration levels. Error bars indicate 95% confidence limits.
dehydration this material displays considerable crack resistance.

3. Notch normal to tubule axis. One alternate orientation was tested at the 0% RH state, that of orientation 2, with the notch oriented normal to the tubular horn. The analysis indicates a significant difference to the parallel oriented samples at the same hydration as well as a significant difference to tubular normal samples at 100% hydration (Table 6). No data are available regarding the location of these samples. The mean value of J for orientation 2 was found to be 75% of the parallel value at 100% RH, while at 0% RH J was determined to be only 48% of the parallel value. This indicates that the process of dehydration has a much greater effect on the relative fracture properties in the direction normal to the tubules (#2) than it does in the direction parallel to the tubules (#1). Similar values were found for most of the other fracture parameters.
Table 6. Results of comparison of dehydrated samples tested at two orientations. S.E. - Standard Error, n - sample number, q - critical extension, Δa - critical crack growth, K - critical Stress Intensity Factor, G - critical Strain Energy Release Rate, J - critical J Integral, orientation as in Fig. 19.

<table>
<thead>
<tr>
<th>Orientation</th>
<th>n</th>
<th>q (S.E.)</th>
<th>Δa (S.E.)</th>
<th>K (S.E.)</th>
<th>G (S.E.)</th>
<th>J (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(x10⁻³ m)</td>
<td>(x10⁻⁴ m)</td>
<td>(x10⁶ N/m³/²)</td>
<td>(x10⁴ N/m)</td>
<td>(x10⁴ J/m²)</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
<td>0.44 (.15)</td>
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<td>3.76 (.82)</td>
<td>1.27 (.46)</td>
<td>8.45 (3.3)</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
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<td>3.45 (2.4)</td>
<td>2.44 (.75)</td>
<td>0.43 (.18)</td>
<td>4.09 (2.8)</td>
</tr>
</tbody>
</table>
DISCUSSION

I. Behaviour of the Fracture Parameters K, G and J.

The design of the mechanical analysis techniques employed in this study allowed a continuous monitoring of the three main fracture criteria as well as apparent crack growth ($\Delta a$). The evaluation of these engineering fracture criteria in the context of the unusual properties displayed by the biomaterial being investigated was a major objective of this study. Some representative examples are shown in this section in order to indicate the relationship between the three fracture criteria and the apparent crack growth during the course of a fracture characterization test.

A. The Stress Intensity Factor, $K$.

The calculation of the stress intensity factor, $K$, is based on several assumptions which restrict the computational analysis to one which is both expedient and, strictly speaking, inapplicable to real materials. Theoretically the material being analysed must be isotropic and linearly elastic. The test specimen must be sufficiently thick to produce a situation of plane strain at the crack tip in order to maintain the stress conditions that the analysis assumes, without interference from boundary conditions. In an ideal material no crack growth
would occur until the critical stress intensity level was reached. At this point crack growth would begin and continue as long as the critical stress intensity was maintained. A plot of $K$ vs. $\Delta a$ would then be a straight horizontal line. In non-ideal materials it is possible for plastic deformation and/or slow stable crack growth to occur. In this case a slight increase in crack length would be evident as the $K$ value increased. Although some crack growth would take place, fracture would not become unstable until the stress intensity level reached a critical value. This critical value need not be indicated by an abrupt change in the $K$ vs. $\Delta a$ curve but a smooth curve indicates an extensive plastic deformation and/or crack damage zone.

The continuous behaviour of $K$ for four samples representing the hydration conditions employed in this study plotted against apparent crack growth is shown in Fig. 26. At the 100% RH level (Fig. 26 A) a smoothly curving, continuously increasing stress intensity value is seen. The critical point determined from the test record 5% slope deviation method is indicated by the large star. The smooth rise of $K$ as a function of crack growth shows slow stable cracking is taking place. This behaviour is indicative of fracture with plane stress in the vicinity of the crack tip (Pook and Smith, 1979). An abrupt change in $K$ vs. $\Delta a$, indicating an unambiguous value of $K$, is not seen for this material until hydration levels of 53% RH or lower (Fig. 26 C & D). At these lower hydration levels unstable fracture was obvious, and the
Fig. 26. Stress intensity factor (K) plotted against apparent crack growth (DELTA a) during a test for representative samples at four hydration levels. A - 100% RH, B - 75% RH, C - 53% RH, D - 0% RH.

Critical failure point determined from 5% deflection of load/displacement record.
determination of the critical $K$ value was not an arbitrary matter. At 75% RH this material was able to resist unstable fracture even at elevated stress intensity levels.

B. Strain Energy Release Rate, $G$.

The strain energy release rate, $G$, is, like $K$, a linear elastic fracture parameter and, therefore, depends on the same basic assumptions. Again, in an ideal material no crack growth should occur until the critical value of $G$ is attained, at which point crack growth would occur as long as the critical $G$ value were maintained. A plot of $G$ vs. $\Delta a$ should, theoretically, yield a straight horizontal line. If plastic deformation or damage is present at the crack tip some apparent crack growth will be evident. The value of $G$ will continue to increase until the critical level of $G$ is reached and unstable fracture will occur. As for $K$, the more abrupt the change in $G$ vs. $\Delta a$, the more confidence can be placed in it indicating a critical failure criterion.

The continuous behaviour of $G$ for the same four samples analysed for $K$ are shown in Fig.27. The 100% RH sample (Fig.27 A) displays a smoothly curving, continuously increasing relationship. This situation is again indicative of fracture in materials under conditions of plane stress in the vicinity of the crack tip (Broek, 1978). The constantly increasing value of $G$ indicates the formation of additional plastic deformation in material at the crack tip as the crack grows stably. This
Fig. 27. Strain energy release rate (G) plotted against apparent crack growth (Δa) during a test for representative samples at four hydration levels. A - 100% RH, B - 75% RH, C - 53% RH, D - 0% RH.

* Critical failure point determined from 5% deflection of load/displacement record.
mechanism accounts for the high levels of strain energy absorbed in the fracture of this material under high hydration conditions.

Fracture will occur in a material when the value of \( G \) is equal to the energy absorbed by the fracture process. In an ideal material, fracture energy is released in the formation of new fracture surface. However, in the presence of plastic deformation, more energy must be supplied to form a new plastic zone before more fracture can occur. The material then appears to become more tough as the crack extends. Unstable fracture occurs when the energy available for crack growth is greater than that necessary to produce new surfaces and create new plastic zones as the crack extends. This will occur when the material is able to achieve high strain energy levels relative to the amount of energy absorbed by the plastic zone. This is the situation seen for the 75% RH sample (Fig. 27B). It was seen in Fig. 24 that this material is capable of attaining high stress intensity levels prior to failure. However, the material is also significantly stiffer at this hydration level (Fig. 14 and Table 1). This greater stiffness means the plastic zone will be smaller at this hydration level. As a consequence of these two factors, more strain energy is stored than is needed for crack growth, and thus, once the critical condition is achieved unstable fracture will follow. The strain energy release rate is lower for this hydration than at 100% RH because less energy is absorbed in plastic deformation in the material near the crack tip. This same unstable fracture
condition is indicated for the lower hydration levels as well (Fig. 27 C & D). The magnitude of $G$ is progressively lower under these latter hydration conditions because the material is again stiffer and would have even smaller plastic deformation zones than that found in the 75% RH sample.

C. The J Integral.

The J integral analysis technique is derived from a path independent characterization of the energy levels around the crack tip. The path independance allows the most convenient contour to be chosen for the determination, i.e. the specimen boundaries. The path independence of the J integral, however, is dependent on the deformation theory of plasticity which does not allow for unloading of the material to occur (Broek, 1978). In cases where large scale plasticity occurs near the crack tip, slow stable fracture will result. During slow crack growth unloading of material behind the crack tip is inevitable. The J integral does not strictly apply to slow stable crack growth and should be restricted only to an analysis of crack initiation.

The shape of the J vs. $\Delta a$ curve has been used to indicate the process of deformation and crack growth in metals (Kobayashi et al, 1979; Landes and McCabe, 1979). It has been shown that the J vs. $\Delta a$ curve for metallic materials displays an initial linear region possessing a relatively steep slope. This corresponds to notch rounding occurring at the notch tip.
prior to true crack growth. Once crack growth is initiated a new relationship between $J$ and $\Delta a$ is seen, and the slope decreases. The point at which the abrupt change in slope takes place and true crack growth begins is used as the material fracture criterion (Fig. 28).

The $J$ values for two specimens tested at 100% RH are shown in Fig. 29 A & B. It is evident that these relationships are not identical to the pattern seen in metals. However, discontinuities are seen which correspond well with the arbitrarily chosen 5% deviation value. The discontinuity in the $J$ vs. $\Delta a$ relationship indicates a change in fracture properties consistently associated with the critical failure point. As mentioned, the meaning of the $J$ integral beyond the crack initiation point is not well understood. At 75% RH the $J$ integral curve had the same general shape as the 100% RH curve (Fig. 29 C). However, the curve did not extend for as long a range, reflecting the lower extensions at which the 75% RH specimens failed. Again, the critical value determined by the 5% deviation method corresponded well with the inflection of the $J$ curve.

At the lower hydration conditions (53% and 0% RH) (Fig. 29 D & E) the $J$ integral appears to take on a more classic form. There are three distinct phases to the fracture process for this type of test: (1) The value of $J$ increases with little or no increase in apparent crack growth, (2) a linear region appearing similar to notch blunting occurs with indications of
Fig. 28. $J$ integral plotted against crack growth and physical interpretation for a metallic material (after Kobayashi et al., 1979).
Fig. 29. J integral (J) plotted against apparent crack growth (Delta a) during a test from representative samples at four hydration levels. A and B - 100% RH, C - 75% PH, D - 53%, E - 0% RH. Critical failure point determined from 5% deflection of load/displacement record.
small scale apparent crack growth, (3) unstable fracture at J values near those determined from critical point estimation. Due to the unstable nature of failure at these hydration levels and the resultant lack of consistent data the true relationship between J and Δa cannot be accurately determined.

D. The Application of Engineering Fracture Parameters to Biological Systems.

The restrictions of linear elasticity and structural isotropy likely invalidate the absolute quantitative values determined for K and G, except possibly at the lower hydration levels. However, this study is internally consistent and, as a consequence, the relative values determined represent a consistent characterization of relative fracture properties under the conditions outlined. The general agreement seen between the several analyses employed combined with the general material properties and morphological evidence support this assertion.

The J integral analysis technique was originally designed in an attempt to extend the applicability of fracture mechanics analysis beyond the linear elastic restrictions and allow for 'reasonable' amounts of plasticity. Although not a widely used technique for the analysis of composite materials, this study indicates that it is quite useful. It yields results consistent with those anticipated, where enough is known about the material to anticipate results (ie. The agreement of the J
integral data and the tensile properties). However, it is likely that the plastic properties of biomaterials in general, and certainly the hoof wall at higher hydrations, take the applicability of the J analysis to its very limits. Taking these limitations into account, the J integral probably provides the best means of characterizing the fracture properties of a material such as hoof keratin. Again, the absolute quantitative values determined may not be reliable in light of the extensive plastic deformation this material is capable of. The relative values are dependable, however, due to the consistency of the comparisons, and as indicated by the consistency of the results.

Absolute characterization of the fracture properties of most materials is a tenuous business and much more so for biomaterials. The techniques applied in the present study have been derived directly from engineering analysis techniques. An attempt has been made to maintain the integrity of the analytical techniques in every manner except basic purpose. The techniques employed were originally designed to characterize the properties of materials under specific mechanical circumstances, for the expressed purpose of quantitatively predicting their behaviour once they have been fashioned into a functional structure. In this study, however, the structure has long since been fashioned, and its functionality proved in better ways than any analytical test could determine. The question posed in this study, especially considering how little is known of this structure or the material from which it is
made, is not 'how much?', as it is in engineering analysis, but simply 'how?'. In what manner and under what conditions do the independent variables analysed affect the growth of cracks in this material? The question of absolute magnitude becomes a secondary feature of the analysis. In this context the analysis employed provides a convenient and adequate method to gain insight into hitherto unrecognized aspects of the functionality of the hoof wall mechanical design.

II. Functional Design of Hoof Wall.

The morphology of the hoof wall indicates that it is a hierarchically organized tissue with important structure-function relationships at several different levels. This discussion will begin with the design of this tissue at the molecular level and work up in scale describing the functional significance of each contributing level. The aim is to indicate the subtle mechanical design features that produce the functional properties of this tissue, itself an integral component of the foot organ.

A. Molecular Design of Keratin as a Composite Material.

Hard keratin, an extremely important structural material in mammals, is universally produced as a composite containing long thin fibres embedded in a partially crosslinked, amorphous matrix. This is not simply an incidental situation, especially
in the hoof wall, because this design can be explained in terms of specific functional requirements. The function of the hoof wall as a contact and supporting structure requires that it possess a reasonable amount of stiffness. Long thin fibres work well in tension but in bending or compression the fibres can slide relative to each other or individually buckle (Fig.1). Materials which must function under compression or bending loads require a matrix which can bind and withstand shear forces between the fibres. In fibreglass the resin functions in this role. The mechanical properties displayed by a composite material can be very different from those of either of the components (Wainwright et al., 1976). These differences can be displayed in both the stress-strain behaviour or the fracture toughness of the material.

Generally, the stiffness of the composite will result from an interaction of the properties of the component phases, the relative proportions of each and the orientation of the fibres relative to the stress. For a given situation of fibre orientation and composition, an alteration in the stiffness of the matrix will be translated into changes in the properties of the material. An extensible matrix will allow the fibres to move relative to each other to a certain degree and the material will be more extensible. The opposite is true for a very rigid matrix.

The fracture behaviour of a composite will also be affected by the fibre and matrix properties and by their
interaction. If the matrix is extensible, fracture energy can be absorbed through deformations between fibres while the rigidity of the material is maintained by the stiffness of the fibres. This allows strain energy to be dispersed to areas away from the location of the crack. Crack growth is inhibited because less energy is available at the crack tip and because the stress concentration is reduced through deformations which distribute the applied loads over larger areas. In addition, another crack stopping mechanism can contribute to the fracture toughness of a composite even if the matrix is quite stiff. If the connection between the fibre and matrix is not too solid, the fibres will separate from the matrix during fracture. In this case the growing crack may spread through the matrix and will be diverted when it reaches the weak interface at the fibre-matrix boundary. The large number of fibres present in a composite means the crack will be diffused into a great many directions absorbing energy and dispersing the stress at the crack tip.

It follows from the above discussion that the mechanical behaviour of a composite material can be determined, to a large degree, by the properties of the matrix. The composite nature of hoof wall keratin and the specific circumstances of its form allow several of its mechanical properties to change as a result of the effect water has on the properties of the matrix. The analyses conducted in this study showed that water content affects both the tensile and the fracture properties of hoof wall keratin. Other studies have indicated a similar response
to hydration for the compression of this material (Leach, 1980; Butler, 1977). The effects of hydration seen in hoof wall are identical to those observed in other mammalian hard keratin materials (see Table 7). The fibres of keratin are composed of stable α-helical chains which would not be expected to change significantly under different hydration conditions. The matrix, on the other hand, acts as a partially crosslinked rubber and has the potential for large alterations in properties dependent on the degree of crosslinking present. Experiments on wool fibres indicate that this is indeed the case. The drying of wool fibres increases the tensile modulus approximately three times, but increases the torsional modulus by a factor of 15 (Feugelman, 1959). Since the microfibrils of wool are strongly oriented parallel to the axis of the wool fibre, this indicates that dehydration affects the properties of the matrix to a far greater degree than it does the fibres (Fraser and Macrae, 1980). Extensive hydrogen bonding between molecules of the matrix is likely responsible for these changes. Such secondary crosslinking would decrease the mobility of the matrix polymers and, in the fully dehydrated state, the matrix properties would approach those of the crystalline fibres. In hoof wall keratin extensive secondary bonding within the matrix would make the material very stiff, a situation seen for the lower hydration state (0% and 53% RH) tensile tests. Greater or lesser levels of hydration would affect the amount of crosslinking present. The level of water content was seen to be inversely related to the modulus over the complete range of hydration (Table 1).
Table 7. Longitudinal elastic moduli of keratinized structures and the effect of hydration level ($10^7$ N/m$^2$). (from Fraser & Macrae, 1980)

<table>
<thead>
<tr>
<th>Material</th>
<th>0%</th>
<th>Relative Humidity</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>Human nail</td>
<td>2.6 (70%)</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>hair</td>
<td>2.3 (70%)</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>stratum corneum</td>
<td>0.19(70%)</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Wool</td>
<td>5.6</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Horsehair</td>
<td>6.8</td>
<td>5.1 (60%)</td>
<td>2.4</td>
</tr>
<tr>
<td>Hoof wall **</td>
<td>14.6</td>
<td>3.36 (53%)</td>
<td>2.6 (75%)</td>
</tr>
<tr>
<td>(parallel to tubules)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Data from this study.
The fracture properties of hoof wall keratin were also affected by the water content of the tissue. It is evident, looking at the J Integral results, for instance (Table 5 and Fig. 24), that dehydration did not cause this material to become excessively brittle. As mentioned, weak interfaces can provide resistance to crack growth in a two phase composite, even when both components are stiff. Crosslinking within the matrix could account for the stiffening of the material as a whole and produce brittle-like behaviour, such as the reduction in crack growth seen at the critical failure point (Fig. 25) and the decrease in strain energy release (Fig. 24) with decreasing water content, without greatly reducing the overall fracture toughness. At the other end of the hydration range, hoof wall tested at 100% RH also provided good crack resistance. Considering the large scale deformations possible for this tissue at this hydration, the ability to absorb energy in the fracture process is very likely due to stress distributing and energy absorbing plasticity near the crack tip. At intermediate hydration (ie. 75% RH) a combination of these two fracture resistance mechanisms is possible. The fibre-matrix interface could still function as a crack stopper, and the extensibility of the matrix (as seen in the 75% RH tensile tests) could allow energy absorption through plastic deformation. Thus, the presence of an intermediate degree of secondary crosslinking might optimize the fracture resistance of this material. This feature is displayed well by the significant increase in the J integral values for the 75% RH tests (Fig. 24). The various
mechanical responses of hoof wall keratin, either as stress-strain or fracture behaviour, can thus be related to the effect of water on the internal bonding in the matrix phase and the interaction between the matrix and the fibres.

Like other epidermal tissues, the hoof wall is the boundary between the dry external environment and the wet, living internal tissues. It has been well documented, both in this study and elsewhere (Baden, 1970; Chapman, 1969; Rigby, 1955; Speakman, 1929; Wildnauer et al, 1971), that hard keratins equilibrate with the hydration conditions of their environment. As an interface between the external and internal conditions, this property produces a hydration gradient within the tissue. This gradient has been measured and, as expected, tissue on the interior is near saturation, and that at the outer surface is equilibrated with the external environment (Leach, 1980).

The effect of hydration on the mechanical behaviour of hoof keratin and the hydration gradient found in vivo in the hoof wall provide a mechanism through which the mechanical properties of different areas of the hoof wall can be adjusted to the requirements of the hoof organ. This is important considering that the mechanical requirements of this tissue are associated with specific locations in the hoof wall and that the tissue moves continuously as new tissue is formed. The tissue, therefore, resides in very different locations during the course of its use, and has very different functions in
these different locations. The hoof tissue which comprises the interior connection to the germinative layer (Fig. 30 A) cannot allow high stress levels to develop or damage will result to the sensitive living cells. The external (Fig. 30 B) and distal (Fig. 30 C) portions must form a hard capsule in order to deal with abrasion forces from the external environment and protect the foot from injury. Most of the wall thickness (Fig. 30 D) must provide the structural integrity to support the animal and resist fracture. At the inner connection layer it is important that stress levels are kept low because this is the attachment point between the hoof wall and the living germinative cell layer. Damage to this cell layer could result in long term disruption of the growth of the hoof wall. This area of the wall is maintained at constantly high hydration levels in the foot because of its close proximity to the underlying vascularized tissues (Fig. 30 E). In this situation the hoof wall keratin would behave similarly to the 100% RH samples tested in this study. Only relatively low stress levels could be achieved before the material began to yield. The extensibility of hoof keratin and the yielding process would allow stresses to be distributed evenly over a large area and kept as low as possible. In locations B and C (Fig. 30) the hoof material will be equilibrated with environmental hydration conditions, which for a terrestrial animal would normally be much drier than the internal tissues. At these positions the hoof wall requires a hard, abrasion resistant covering to protect against puncture and excessive wear. The rigidity of
Fig. 30. Inter-relationships of tissues in the hoof wall.
A - stratum internum
B - external surface
C - distal contact surface
D - stratum medium
DI - dermal/epidermal interdigitation at 1° and 2° lamellae
LG - living germinative layer both at the coronary border and along the dermal/epidermal interdigitation
E - collagenous connection between epidermal wall and skeleton. Area containing vascularized tissue.
hoof keratin at hydrations below 53% RH indicate it could possess these properties in these locations. The majority of the hoof wall (Fig.30 D) would be required to provide the structural integrity of the wall. Considering the size and speed of the horse, a reasonably high degree of stiffness is needed but this must also be balanced with the ability to resist fracture. These are the properties which appear to be optimized by hoof keratin at 75% RH (Figs.14 and 24). Leach (1980) found that the major portion of the wall midway between the internal and external surfaces of the hoof had water contents of between 17-24% water by weight. This is the same level determined for the 75% RH samples tested in this study (18.13% 0.24 s.d. n=23).

Thus, hoof wall keratin must have different mechanical properties at different locations. These tissues remain as part of the wall for approximately a year and grow distally continuously. Therefore, mechanical properties must be adjusted over time as well. Recall that epidermal tissues function after the cells have undergone a programmed death, and thus, the tissue is unable to physiologically adjust its components to the varying requirements. The capacity for preprogramming the material to have different mechanical properties at different hydration levels allows this one material to fulfill all the mechanical roles necessary for the optimum functioning of the hoof organ. The modulation of the secondary bonding forces within the matrix polymers alters the mechanical properties of the entire tissue and appears to play a major role in the
adjustment of the tissue properties.

One aspect of this situation not looked at specifically in this study is the capacity of the stratum internum to act as a 'shock absorber' for the internal germinative layer. The connection of the hoof wall to the underlying dermis along the inside surface (Fig.30 A) is accomplished through a large number of convolutions termed primary lamellae (Fig.3). The primary lamellae are themselves connected to lamellae of the dermis through an interdigitation of much smaller secondary lamellae oriented perpendicular to the primary lamellae (Fig.3). This system increases the surface area of the connection and, therefore, reduces the stresses encountered by the living, connecting layer. The experiments conducted in this study indicated that the hoof keratin surrounding this connection region has the ability to yield and absorb considerable amounts of energy at low stress levels. This suggests that the internal hoof keratin is admirably suited to act as a final 'safety valve' to protect the very important connection between the hoof wall and the remainder of the skeletal system. It would be interesting to know if this material could recover its original mechanical capabilities once it was strained beyond the yield point and, if so, to what degree and under what conditions this would occur.

The experiments performed in this study are of a preliminary nature and do not characterize this material fully. The subtle control of various mechanical properties through
specific responses to specific hydration conditions has been demonstrated. The determination of the precise molecular mechanism responsible for this control and the limits to it are beyond the scope of this study. It would, nevertheless, be interesting to know under what conditions, and why, the change in fracture properties observed between 100% and 75% RH tests and 75% and 53% RH tests occurred. It is likely that very different mechanisms are operating to produce the effects seen at 100% and 0% RH, and that the properties observed for 75% RH represent a combination of both.

B. The Role of the Cellular Architecture

All epidermal tissues are formed on a basal membrane which connects to the underlying dermal component of the integument. The cells are produced in association with this membrane and are replaced by newly forming cells as the tissue grows. This mode of growth places certain constraints on the cellular organization possible within these tissues. The epidermis of skin (stratum corneum) is formed in this manner, resulting in the cells being organized in a plane parallel to the basal membrane. This arrangement will produce a plane of weakness along which cracks can pass relatively easily. Binding the cells firmly together can reduce this effect, but unless some component is oriented perpendicular to the crack plane, a crack could spread with little obstruction. The experiments studying the effect of the orientation on the fracture
properties of the hoof wall showed that the intertubular material dominated the path of crack growth and that this occurred because, in terms of fracture energy, the cost of crack growth was significantly less in the direction of the intertubular orientation than in all other directions (Figs. 20 and 21). The intertubular material is formed on a flat basal membrane in a manner similar to stratum corneum. Consequently, the intertubular material forms a plane of fracture instability. This organizational pattern might allow layers of the wall material to be broken off, producing a severe limitation to the functional capabilities of the hoof wall. The solution is to orient a structural component perpendicular to the plane of weakness, and this component is provided by the tubular material.

Because keratinocytes can only be produced parallel to the basal membrane, it is necessary to modify the arrangement of the basal membrane in order to produce cells oriented perpendicular to the intertubular material. Through the simple outpouching of the basal membrane to form the dermal papillae, the 'growth plane' for the formation of the tubule cells becomes oriented perpendicular to the intertubular material. Cells are produced on the sides of the papillae which have orientations perpendicular to the intertubular material, and as a result of the fracture discontinuity they present, a material possessing reasonable fracture stability in all three directions is created.
One advantage of reinforcing the planar intertubular material with tubules is the lack of connection between these tubules. A crack running along the orientation of the intertubular material must pass through many tubules, but a crack running along a tubule must pass through a continuum of intertubular material. Of course, the most important direction, in terms of maintaining the integrity of the hoof wall, remains the vertical direction. The evidence presented in this study shows that the intertubular material provides an impressive resistance to fracture in any direction with a vertical component (Fig. 20).

The mammals have developed an amazing diversity of hard keratin appendages. These take on an extremely wide variety of forms to accomplish an equally wide range of functions. Among these are hooves, claws, horns of various types and even the baleen of whales. An interesting feature of all these structures is the presence of tubular structures, produced on dermal papillae, embedded in intertubular material, (Ryder, 1962; George, 1956). The diverse functions of these appendages suggest that they are not homologous and, therefore, arose independently. This supports the assertion that this morphological organization requires only a minor alteration in the basal membrane and suggests that the structural organization has an important functional significance. It is quite possible, considering what has been determined here regarding the hoof wall, that the tubular organization is a reinforcement which adds to the fracture toughness and wear
properties of these diverse epidermal appendages.

The final goal of a study such as this is the interpretation of the data in terms of the function of the animal concerned. As has been seen, design at the molecular and cellular levels can have significant effects on the properties of a material such as this. It is necessary, however, to show how these effects apply to the animal when the structure investigated is a functioning whole. As yet, the exact morphological relationship between the tubular and intertubular material throughout the entire hoof is not well known. The tubules are always parallel to the external surface of the hoof and run continuously from the proximal germinitive layer to the distal contact surface. In general, the intertubular material runs somewhat perpendicular to the tubular axis. In light of the evidence presented, and considering this tissue in strictly a material sense, it may seem that the most efficient design would have the tubules and intertubular material oriented 90 degrees to each other. It was seen, however, that the mean tubular-intertubular angle was approximately 55° to 60°. The samples used in this study were taken from the toe region of the hoof wall. It is interesting to note that in a normal horse the hoof wall strikes the ground at an angle of approximately 55° for the front feet and 50° in the rear feet. If the intertubular material were oriented at 90 degrees to the tubules in the toe region, the weakest fracture direction would be oriented to allow the end of the wall to be broken off (Fig. 30). An angle of between 55° and 60°, however, places the
Evidence has been presented which suggests that the most distal portion of the hoof wall can suffer from internal damage which reduces the fracture resistance capabilities of the tissue (Fig. 18 and Table 2). The reduction of fracture toughness in a composite as a result of use is generally due to the production of microscopic cracks within the material. As has been discussed, cracks in a material under stress can lead to larger cracks and finally to fracture. It is then advantageous to remove flawed material. It would appear that the hoof wall is admirably designed to do this. The congruency found between the weakest fracture plane in the hoof wall and the ground contact surface strongly suggests that the wall is designed for a degree of controlled wear while at the same time guarding against fracture in any vertical direction. The hoof keratin functions as part of the hoof wall, and in a non-living state, for approximately one year. For the purpose of eliminating flaws in old tissue, controlled wear can be advantageous as long as it does not exceed the rate at which replacement tissues can be generated conveniently. The control of the plane of wear would also be important in determining the contact of the foot and the ground. The equine leg is a finely balanced locomotory structure which depends on dealing with specific stress conditions. It is important to the proper functioning of the rest of the leg that the manner in which the foot strikes the ground remain consistent.
This study has shown that it is possible to employ some rather abstract or esoteric analysis techniques to determine the subtle functional design strategies in the hoof wall. The fibre-matrix composite design gives rigidity and toughness to this material and, through a specific response of the matrix polymers to hydration, allows these mechanical properties to be altered to the requirements of specific areas of the wall. The cellular architecture is dependent on the mode of growth in this tissue. The development of dermal papillae with germinative membrane oriented perpendicular to the natural plane of weakness decreases the effect of the weakest orientation and balances, to some degree, the fracture toughness of the material. The relationship between the weakest plane of fracture and the ground contact surface allows controlled wear to occur and an appropriate hoof shape to be maintained.

These important biological conclusions were determined by analysing the tissue as if it were a simple engineering material. It is important to remember, however, that, unlike engineering materials which are designed as materials and later used to build structures, structural biomaterials are designed, produced and used in terms of the structural organ they compose. With a material such as hoof wall, it is very difficult to isolate the material from the structural properties. Sections removed from the hoof organ to act as test samples cannot represent the total interactive properties of the organ. Considering the complexity of this biological
structure, it is very pleasing to see how much biological insight can be provided by an abstract, engineering analysis.
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