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Measurement of the fracture toughness of extensible connective tissues

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A tear test was found to be suitable for determining the fracture toughness of two extensible connective tissues: rat skin and the mesogloea of the sea anemone *Metridium senile*. This technique allows the mapping of toughness variations over an area of rat skin. The toughness bears an inverse relationship to maximum stiffness parallel to the direction of crack propagation. Toughness of rat skin increases slightly with increasing extension rate.

1. Introduction

Animal connective tissues are pliant composite materials consisting of high modulus fibres embedded in an amorphous matrix. In most cases the fibres are principally collagen and the matrix consists of highly hydrated proteoglycan (protein–polysaccharide complex) gel [1]. The prime function of these collagenous tissues is mechanical; skin, whilst having other important physiological properties, primarily provides support and protection to underlying structures. Tendons, ligaments and blood vessel walls have obvious mechanical roles. In animals such as the sea anemone the connective tissue (mesogloea) in the cylindrical body wall provides a container for the hydrostatic skeleton.

The connective tissues, especially the highly extensible ones such as skin, mesogloea and artery wall are extremely successful in avoiding fracture, despite the often quite considerable stresses acting upon them. Often, in tendons and ligaments, the avoidance of catastrophic rupture in a sub-critically damaged tissue can be achieved by behavioural adaptation to a sensing of damage; e.g. pain from an injured tendon inhibits full locomotory activity. However, there is no obvious means of resting a damaged major blood vessel. Neither would a tear or cut introduced into the skin over a knee joint be expected to propagate catastrophically under the loads imposed on it by the animal running about. This success in avoiding rupture in normal circumstances is highlighted by pathological conditions in which the connective tissues are defective. The formation of cerebral aneurisms (bulges in the artery wall) often ends fatally when the aneurism bursts [2]. Connective tissue disorders such as lathyrism [3] and some forms of Ehlers–Danlos syndrome [4] which render normally tough connective tissues brittle can often result in death due to bursting of a major blood vessel.

The ability of a material to resist the propagation of a crack through it is measured in terms of its fracture toughness. The aim of this work is to assess a possible method for measuring the toughness of extensible connective tissues that can typically be deformed by 50 to 100% or so [5] before breaking.

1.2. Fracture mechanics approach

Linear elastic fracture mechanics (LEFM), founded on the work of Griffith [6], describes the toughness of a material in terms of a critical strain energy release rate, G_c, at which a crack of a given size will just start to grow under the influence of a given stress field. The value of G_c defines the balance between the processes of strain energy release from the body of the specimen and energy consumption by processes associated with the creation of new fracture surfaces as the crack just starts to propagate. LEFM analyses are constrained by the assumptions of low-amplitude strain and

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linear elasticity, two conditions that do not apply to the extensible connective tissues. One further complication is the variability of connective tissue specimens both from animal to animal and from different sampling sites on one animal. Thus any compliance calibration type of fracture toughness measurement [7, 8] is not useful because of the difficulty in obtaining many specimens with identical properties.

Rivlin and Thomas [9] and subsequently Greensmith and Thomas [10] have shown that a tear test can be used to measure the critical strain energy release rate of rubbers, a class of materials that also show finite strain, non-linear elasticity. The specimen geometry and load–deformation behaviour are shown in Fig. 1.

Rivlin and Thomas [9] derived the following expression for the critical strain energy release rate in general terms, calling it T_{ch} to distinguish it from G_c's calculated by other means.

$$T_{ch} = \frac{W_0 A_0 - 2\lambda F_0}{t_h} \quad (1)$$

where t_h is the specimen thickness, F_0 the plateau tearing force (see Fig. 1c), λ the extension ratio in the "arms" of the specimen at F_0, W_0 the strain energy density in the arms of the specimen at F_0, and A_0 the cross-sectional area of the test piece arms.

For rubbers, W_0 can be calculated from an expression relating stored strain energy to the strain invariants. However, by directly measuring W_0 from load–entension tests on un-notched specimens, Equation 1 can be used to measure T_{ch} on connective tissue.

Rivlin and Thomas [9] also note that, as the stress in the arms of the test piece is usually low, $\lambda \cong 1$ and $W_0 \cong 0$, so that a fair approximation is given by;

$$T_{ch} = \frac{-2F_0}{t_h}. \quad (2)$$

Another useful feature of this fracture technique is that, because of the specimen geometry, the fracture propagation is slow and controlled (quasi-static) [11, 12] and can be stopped at any point by unloading the specimen. In this case the strain energy being supplied is approximately equal to that required to make new surfaces, and

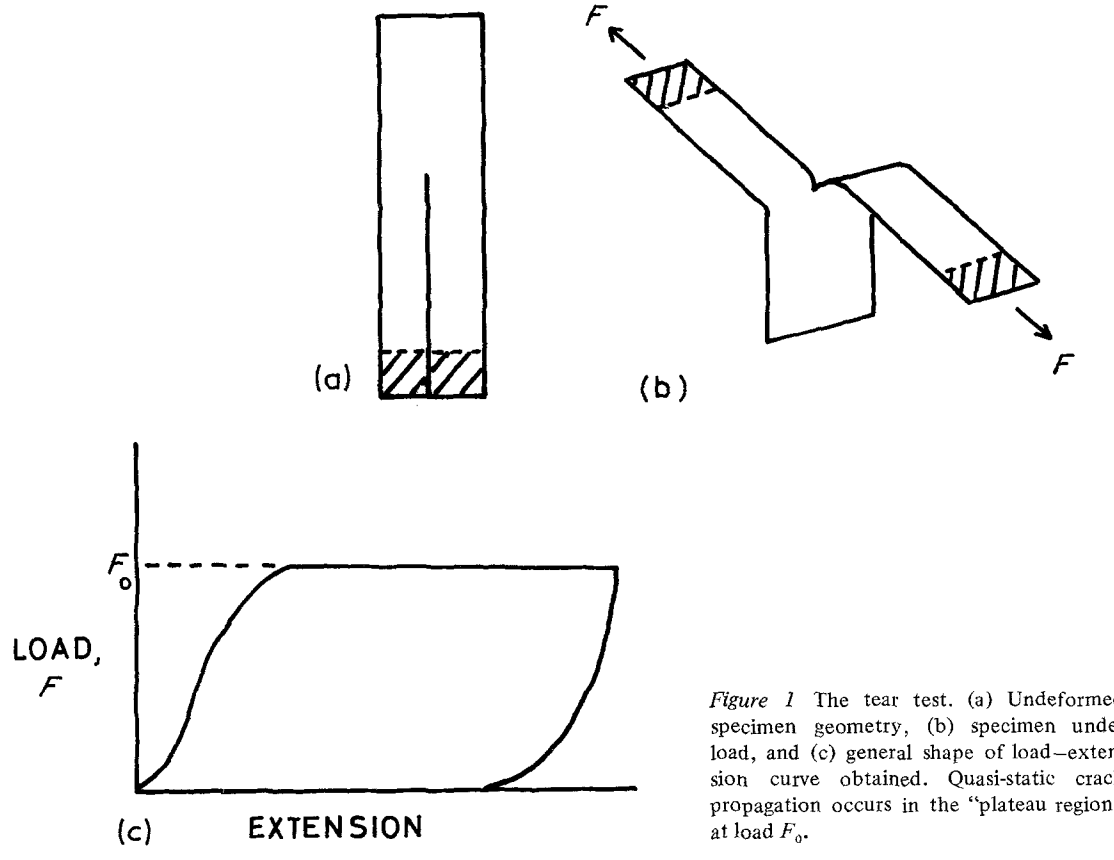

Figure 1 The tear test. (a) Undeformed specimen geometry, (b) specimen under load, and (c) general shape of load–extension curve obtained. Quasi-static crack propagation occurs in the "plateau region" at load F_0.

so the strain energy release rate, G, at any point in the propagation is close to the critical case, G_c. As the total work, U, expended in propagating a crack of length a_1, to a new length a_2 is given by:

$$U = \int_{a_1}^{a_2} Gt_h \, da$$

and $G \cong G_c$ at all stages

$$U = G_c t_h (a_2 - a_1).$$

$t_h(a_2 - a_1)$ is the area through which the crack has been propagated, A_p, and so

$$G_c = \frac{U}{A_p}. \quad (3)$$

The result from this method of calculating G_c shall be termed γ_T to distinguish it from T_{ch}. γ_T is a work of fracture measurement, but usually work of fracture is expressed as total work done in relation to the area of new surfaces created, which is twice A_0. By expressing γ_T in terms of work done per unit area cleaved by the propagation, the numerical value is directly comparable to other G_c measurements, and this practice is adopted here.

In this paper the applicability of tear testing to the measurement of toughness is assessed. Both T_{ch} and γ_T can be calculated from individual specimens and the degree of agreement between them should indicate the validity of the approach. The effect of extension rate and temperature on toughness values are also examined, and variability in results with specimen position considered.

2. Materials and methods

2.1. Specimen preparation

2.1.1. *Metridium senile* mesogloea

Sea anemones were obtained from the Marine Biological Association laboratories at Plymouth and the Universities of London and Glasgow laboratories at Millport and kept in circulating sea water tanks for up to two months. Animals were anaesthetized in a 50:50 mixture of 20% $MgSO_4$ and sea water [13, 14] for 3.5 to 4 h. During the last 3 h this mixture was delivered into the enteron of the animal via a hypodermic needle at 2 ml min^{-1}. Strips were then cut from the cylindrical body wall and lightly scraped to leave a thin sheet of mesogloea. Specimens of the shape shown in Fig. 1 were cut from this and the orientation of crack propagation with respect to the cylindrical body axis noted.

2.1.2. *Rat skin*

6 to 8 week old albino rats of the Charles River strain were fed for 4 weeks *ad libitum* on MRC diet no. 86. The 10 to 12 week old animals were killed by a blow to the back of the neck and the skin was shaved before removal of a band of skin between the pelvic and pectoral girdles. This band was cut along the mid-ventral line and taken off as a flat sheet which was stored frozen at −20°C until use, when it was thawed in saline solution and cut into specimens of the shape shown in Fig. 1. The direction of crack propagation was arranged to run either parallel to the longitudinal body axis, or circumferentially.

2.2. Test method

Tear tests were carried out on an Instron tensiometer model 1026 at room temperature, using a constant extension rate of 50 mm min^{-1}. The specimens were unloaded at the same rate before fracture had propagated along the full length of the test piece. Mesogloea specimens were glued with cyanoacrylate between screwed-together clamp plates and tested under the anaesthetic mixture in a tank attached to the Instron. Rat skin specimens were held in pneumatic grips and tested wet, in air. After testing, rat skin test pieces were allowed to recover for 4 h and simple unnotched strips then cut from one arm of the specimen. These were extended at the same rate of 50 mm min^{-1} and the extension ratio and strain energy at the plateau tearing force for that specimen noted. T_{ch} and γ_T were evaluated for each specimen as outlined above.

2.3. Effect of extension rate

In order to assess the effect of different extension rates on the measured values of G_c, a rat skin was prepared as above and divided into longitudinal specimens only. These were assigned to three groups, the spread of specimen positions over the area of the skin being as even as possible. The groups were subjected to tear testing and analysis as above except that one group was tested at an extension rate of 5 mm min^{-1}, one at 50 mm min^{-1} and one at 500 mm min^{-1}. A typical tear test was completed in about 3 min.

2.4. Effect of temperature

A rat skin was prepared above and divided into circumferentially propagating specimens, which were allocated to three groups, each group to be tested

at a different temperature. Positional spread from group to group was as even as possible. Tear testing was carried out at a constant extension rate of 5 mm min^{-1} on a tensiometer described by Vincent [15]. This machine is small enough to be easily transported to and equilibrated in constant temperature rooms set at 7, 23 and 30.5° C. Specimens were equilibrated to these temperatures before testing.

3. Results

3.1. *Metridium senile* mesogloea

A typical load–extension curve from a tear test on mesogloea is shown in Fig. 2a. The plateau region is not smooth, but shows some oscillation about some mean value, a behaviour known as "stick-slip" tearing [10]. Using a mean plateau tearing force value, T_{ch} was calculated using Equation 2, with the results shown in Table I together with γ_T calculated on the same specimens. Values for longitudinal crack propagation fall within the range obtained for circumferential propagation.

3.2. Rat skin

A typical load–extensions curve from a rat skin tear test is shown in Fig. 2b. As with mesogloea there is some oscillation of load in the "plateau" region but at a much higher mean level than for mesogloea. Mean T_{ch} and γ_T values from a typical skin are shown in Table II. Because of the greater compliance of this material the approximate formula (Equation 2) for T_{ch} gives an appreciably low result, this is shown as T_{ch} (approx) in Table II together with the corrected value (using Equation 1) for comparison. There is a marked difference between values for circumferential and longitudinal being more difficult.

TABLE I Toughness of *M. senile* mesogloea

Crack orientation	T_{ch} (kJ m^{-2})	γ_T (kJ m^{-2})
Circumferential	1.21 ± 0.10 (15)	1.14 ± 0.11 (15)
Longitudinal	1.22 ± 0.06 (3)	0.94 ± 0.08 (3)

Mean value ± one standard error (number of samples).

As well as variations in overall toughness from animal to animal, there is a considerable variation in toughness from site to site over the area of skin examined. Fig. 3 shows this distribution; whilst longitudinal toughness is in general higher than circumferential for corresponding positions either side of the mid-dorsal line, both longitudinal and

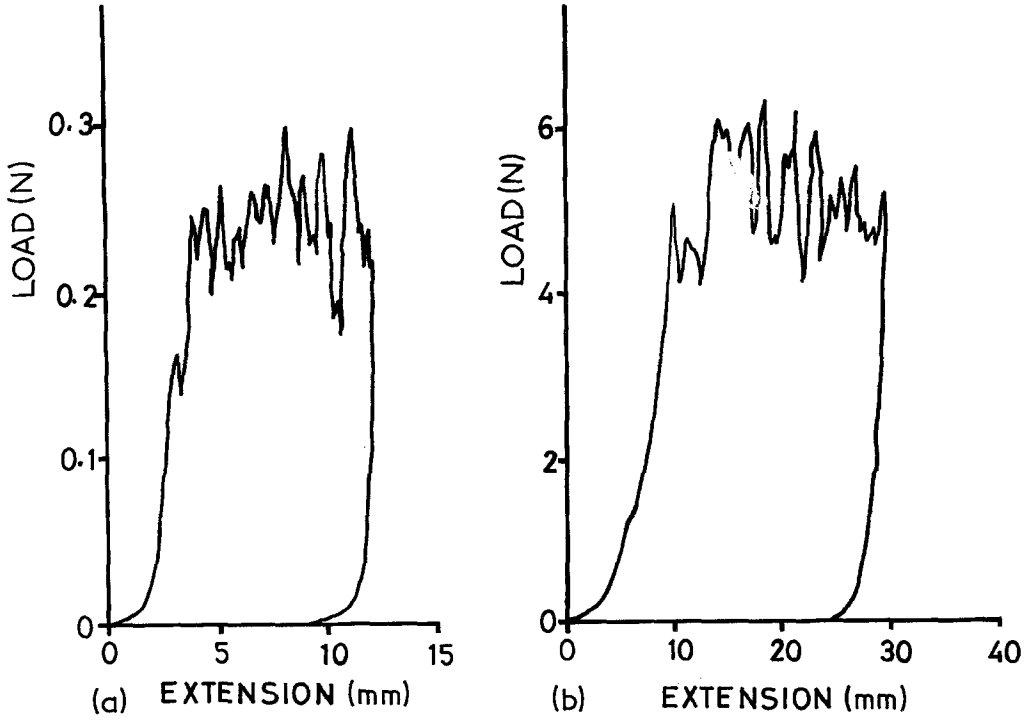

Figure 2 Load–extension curves for tear tests on (a) mesogloea, and (b) rat skin. Note the change in axes between (a) and (b).

TABLE II Toughness of rat skin

Crack orientation	$T_{ch(approx)}$ (kJ m^{-2})	T_{ch} (kJ m^{-2})	γ_T (kJ m^{-2})
Circumferential	11.08 ± 1.68 (8)	13.7 ± 2.29 (8)	13.2 ± 1.79 (8)
Longitudinal	16.53 ± 1.34 (8)	22.6 ± 1.84 (8)	26.9 ± 2.73 (6)

Mean value ± one standard error (number of samples).

circumferential toughness decrease ventrally. From the load deformation curves for unnotched strips prepared for strain energy assessment it is possible to measure the maximum stiffness in the direction of crack propagation exhibited by specimens from the same positions on the skin: these are shown in Fig. 4.

3.3. Effect of extension rate

Fig. 5 shows the mean toughness ± one standard error for each group of skin specimens at the three extension rates used. Toughness can be seen to approximately double when the extension rate is altered by two orders of magnitude.

3.4. Effect of temperature

Fig. 6 shows the mean toughness ± one standard error for each group of skin specimens at the three temperatures studied. Mean toughnesses at 7 and 23° C are no different, and there is some suggestion that the toughness may increase at 30.5° C, although this is not statistically significant.

4. Discussion

Tables I and II show good agreement between T_{ch} and γ_T for both mesogloea and rat skin. Thus the technique shows reasonable internal consistency. The small discrepancies between the two methods of calculating G_c may be due to the fact that these

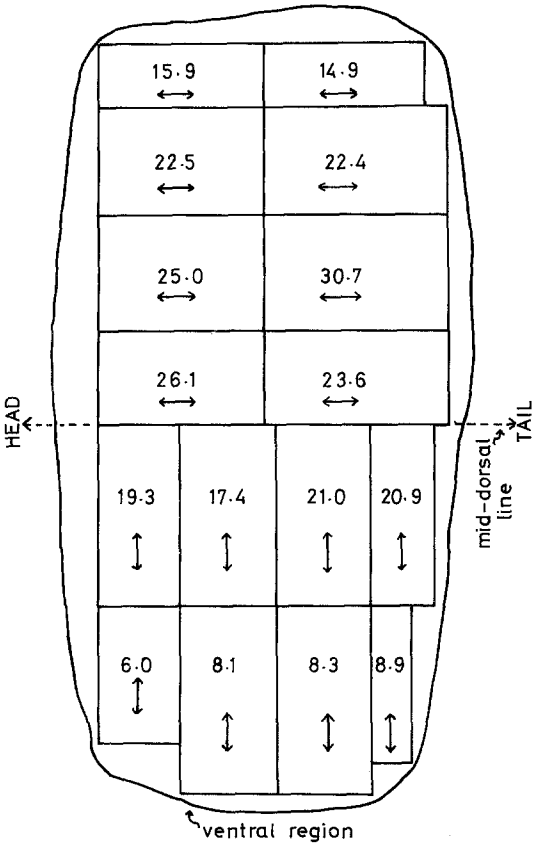

Figure 3 Toughness of various areas of rat skin. Figures denote T_{ch} values in kJ m^{-2}. Arrows indicate direction of crack propagation. Extension rate = 50 mm min^{-1}.

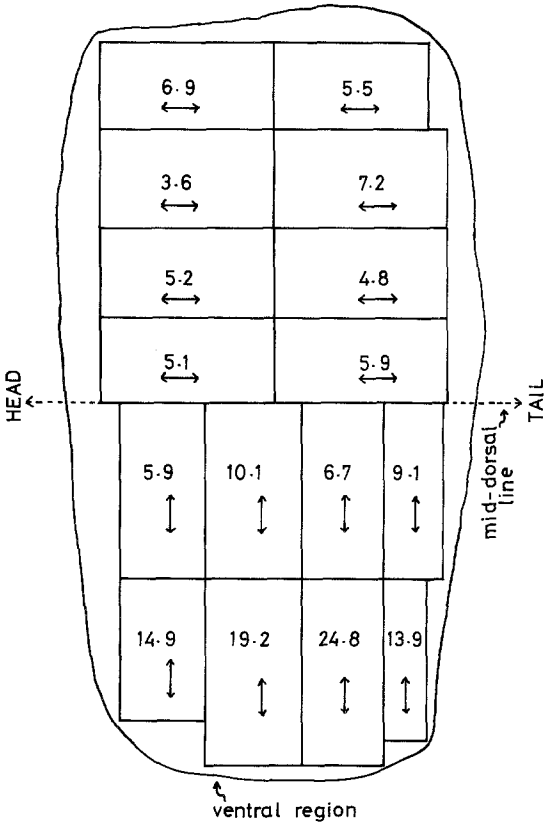

Figure 4 Variation of stiffness over a rat skin. Figures denote maximum incremental stiffness in MN m^{-2}. Arrows indicate direction of testing. Extension rate = 50 mm min^{-1}.

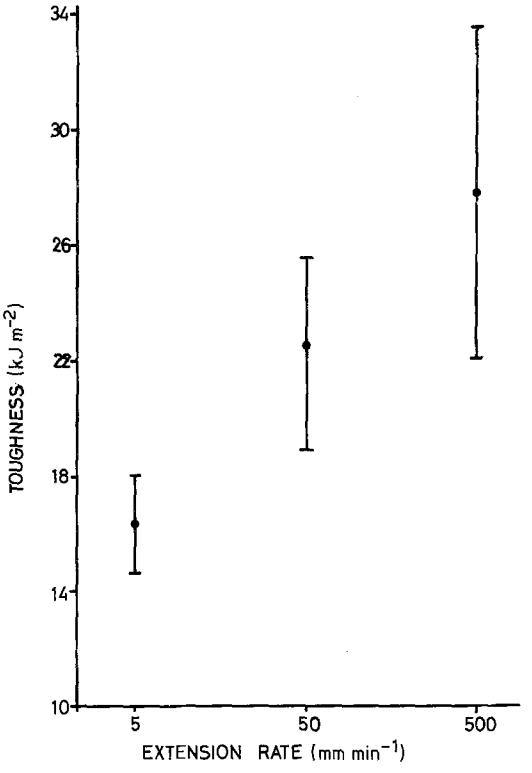

Figure 5 Mean toughness (γ_T) against extension rate. Error bars represent ± one standard error.

materials exhibit hysteretic losses, whereas Rivlin and Thomas' formulation (Equation 1) assumes perfect elasticity. Rat skin differs from mesogloea in that it appears that the approximate formula for T_{ch} (Equation 2) gives an appreciably depressed value. This is because of the relatively high compliance of skin; although the stress generated in the arms of the test piece for W_0 to remain negligible, the extension ratio (assumed $\cong 1$ in the approximate formula) is in the region of 1.2 to 1.5 at propagation. Toughness values obtained for both mesogloea and rat skin are in broad agreement with values obtained from a limited number of pure shear fracture tests [16]. The jagged nature of the load–extension curves for both materials in the plateau region is reminiscent of that for paper [16] and may well be an expression of the fibrous nature of the composite. No fibre pull out of the type described by Kelly [17] is observable on examining the fracture surfaces of mesogloea or skin, but delamination or defibrillation of large collagen fibre bundles into smaller units as a response to stresses near to failure has been shown in cyclically loaded connective tissues [18] and may be an energy dissipation mechanism that contributes to the toughness of these materials. Absolute values of toughness obtained are not especially great; mesogloea at about 1 kJ m^{-2} is roughly as tough as bone, but skin is an order of magnitude tougher and at about 10 kJ m^{-2} is of the same order of magnitude as wood [19].

The technique of tear testing is advantageous in that it yields a value for toughness for each specimen used and does not require the use of supposedly identical specimens of other methods [7, 8]. Furthermore, specimen size can be reasonably small, as there are no large gripping requirements. These advantages facilitate the mapping of variations over an area of material, such as shown

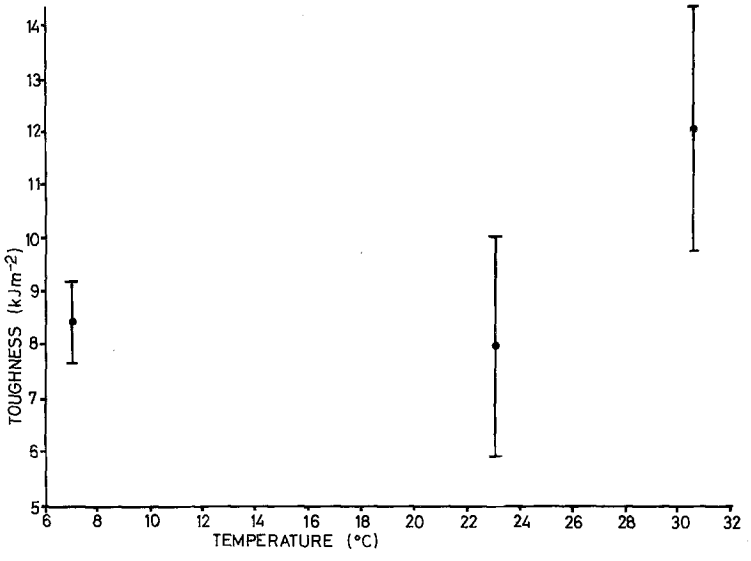

Figure 6 Mean toughness (γ_T) against temperature. Error bars represent ± one standard error.

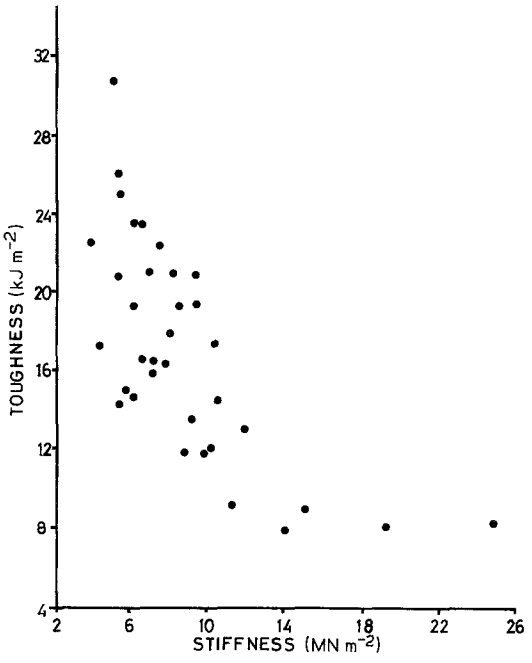

Figure 7 Toughness against maximum stiffness for rat skin pieces.

in Fig. 3. Biologically it may be important to possess tougher skin dorsally; animals attacked by predators usually present their backs (dorsal surface) rather than expose the "soft underbelly" (ventral region). It is interesting to note that variations of toughness with orientation and position are inversely mirrored by limit stiffness (Fig. 4). Fig. 7 shows the toughness from a collection of rat skin tear tests plotted against limit stiffness parallel to the propagation direction. Some form of inverse relationship is clearly apparent. These variations in stiffness and toughness may be ascribed to varying preferential orientations of collagen fibres in different areas of the skin. Variations of this nature are mapped on human skin by the well-known Langers lines [20–22], or directions of minimum extensibility. Similar variations in stiffness in tanned sheep skin have been observed by Poulter and Ward [23]. This inverse relationship between stiffness and toughness may be explained by considering that if there is a preferred orientation of fibres in any one direction then the stiffness in that direction will be higher due to the reinforcing effect of the fibres on the matrix. Conversely, propagation of rupture along that direction would be easier because the crack would tend to run parallel to a greater proportion of fibres rather than having to cross them.

Toughness values for rat skin show dependence on extension rate and temperature, although the changes in toughness over the ranges studied are small. It is interesting to note that toughness increases with increasing extension rate but remains roughly constant with increasing temperature and, if anything, may possibly increase at the highest temperature studied. Work on time and temperature effects on viscoelastic materials [24, 25] shows that properties such as stiffness that increase with extension rate generally decrease with temperature, in contrast to the observations on toughness here. Structural changes in the proteins of the tissue with temperature, such as their degree of hydration [26] may be producing effects which prevent the normal time–temperature correspondence being seen.

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