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Elastic proteins: biological roles and mechanical properties

John Gosline[1*], Margo Lillie[1], Emily Carrington[1,2], Paul Guerette[1], Christine Ortlepp[1] and Ken Savage[1]

[1]*Department of Zoology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4*
[2]*Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881, USA*

The term 'elastic protein' applies to many structural proteins with diverse functions and mechanical properties so there is room for confusion about its meaning. Elastic implies the property of elasticity, or the ability to deform reversibly without loss of energy; so elastic proteins should have high resilience. Another meaning for elastic is 'stretchy', or the ability to be deformed to large strains with little force. Thus, elastic proteins should have low stiffness. The combination of high resilience, large strains and low stiffness is characteristic of rubber-like proteins (e.g. resilin and elastin) that function in the storage of elastic-strain energy. Other elastic proteins play very different roles and have very different properties. Collagen fibres provide exceptional energy storage capacity but are not very stretchy. Mussel byssus threads and spider dragline silks are also elastic proteins because, in spite of their considerable strength and stiffness, they are remarkably stretchy. The combination of strength and extensibility, together with low resilience, gives these materials an impressive resistance to fracture (i.e. toughness), a property that allows mussels to survive crashing waves and spiders to build exquisite aerial filters. Given this range of properties and functions, it is probable that elastic proteins will provide a wealth of chemical structures and elastic mechanisms that can be exploited in novel structural materials through biotechnology.

Keywords: elastic proteins; mechanical design; elastin; collagen; byssal fibres; spider silks

1. INTRODUCTION

The objective of this symposium was to develop an understanding of structural design in elastic proteins, to elucidate the functional role that these materials play in the lives of real organisms and to discover whether molecular mechanisms in these materials could be exploited through biotechnology. One striking feature of the elastic proteins we consider is that they exhibit an exceptionally broad range of material properties and functional roles. As a starting point, therefore, it may prove useful to explore some general features of mechanical design in elastic proteins to set the stage for the detailed analysis of the individual proteins in the sections that follow.

It is frequently assumed that mechanical and biochemical devices in organisms represent perfect or near perfect solutions to the problems that organisms encounter in their lives. Although it is not clear if this optimistic view is strictly true for elastic proteins, or for any other systems in biology, it is likely that elastic proteins are relatively well designed because they have been tested and modified through aeons of evolutionary history. For the purpose of our discussion, we will define 'design' as the relationship between the structure and the function of biological devices, as they exist in living organisms. There are two paths towards an understanding of the design of elastic proteins. Most obvious is that the direct analysis of microscopic and molecular structure will reveal the details of molecular mechanisms in elastic proteins and will document structure–property relationships for these materials. This approach, however, only takes us half way to an understanding of the design. The problem is that material properties alone do not specify the function of a mechanical device. Materials science offers a large number of properties that we can use to quantify the behaviour of a structural material, but to understand the function of a device it is necessary to identify which of the properties are key to its design. Thus, it is essential that we are able to evaluate quality in light of the material properties that truly reflect the function of elastic proteins in living animals. It is this second path, the evaluation of material properties in the context of the mechanical role, which forms the substance of this paper.

2. MATERIAL PROPERTIES

Table 1 provides a convenient way to begin thinking about the problem at hand. It lists a number of functional attributes that can be assigned to structural materials and gives the associated material properties and units that can be used to quantify these attributes. Each of the attributes can be assessed by a mechanical test, usually some variation of a stress–strain test, and the key to evaluating design quality will be to decide which properties are dominant in the function of particular elastic proteins in life.

Figure 1 shows the results of mechanical tests carried out on a number of elastic proteins, proteins unfortunately

* Author for correspondence (gosline@zoology.ubc.ca).

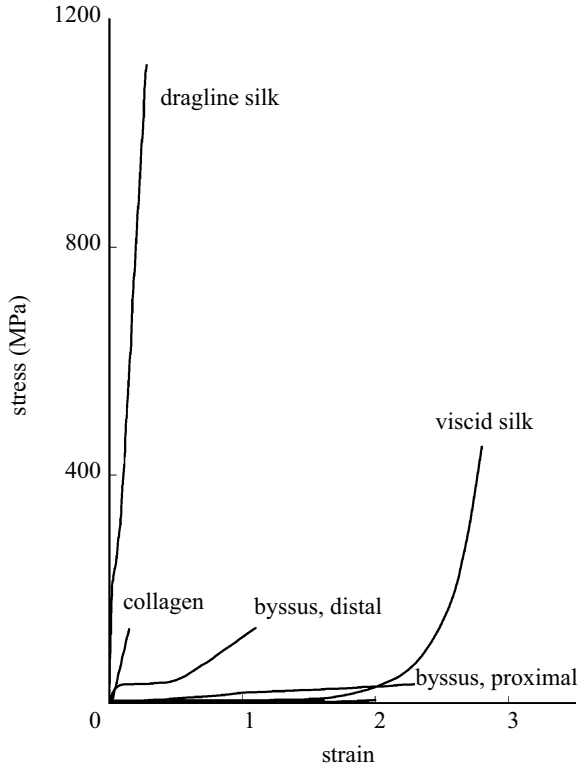

Figure 1. Stress–strain curves for seven elastic proteins. The plot is dominated by super-strong spider silks; as a consequence, the curves for the rubber-like proteins, elastin and resilin, are indistinguishable from the strain axis and have been left unlabelled. The sources for these stress–strain curves are listed in the legend to table 2.

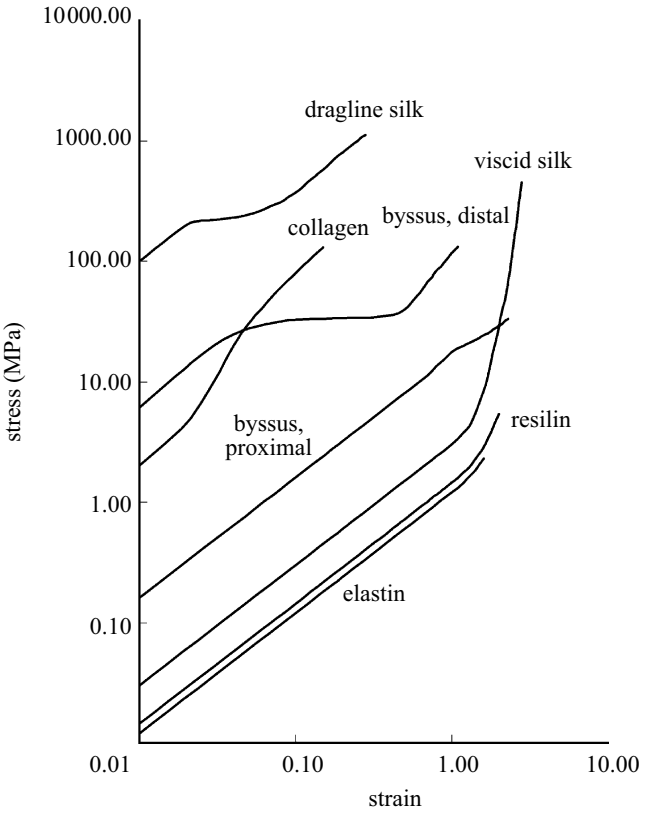

Figure 2. Stress–strain curves for the seven elastic proteins from figure 1 are plotted on logarithmic axes to reveal the full diversity of their mechanical properties.

Table 1. Some functional attributes of materials and the material properties and units used to quantify these attributes.

functional attribute	material property	units
stiffness	modulus of elasticity, E_{init}	Nm^{-2}
strength	stress at fracture, σ_{max}	Nm^{-2}
toughness	energy to break work of fracture	Jm^{-3}, $J\,m^{-2}$
extensibility	strain at failure, ε_{max}	no units
spring efficiency	resilience	%
durability	fatigue lifetime	s to failure or cycles to failure
spring capacity	energy storage capacity, W_{out}	Jkg^{-1}

restricted to those that can be obtained as macroscopic samples and tested by conventional methods. The mechanical tests were carried out at temperature and hydration states that correspond to the *in vivo* conditions for the proteins. That is, spider silks were tested in air, byssus fibres were tested in seawater and tendon collagen, elastin and resilin were tested in water or dilute physiological saline. The stress–strain curves plotted are typical data that would be seen in a constant strain-rate test to failure.

It is clear from figure 1 that elastic proteins are remarkably diverse in their properties, but unfortunately, the stress–strain curves in figure 1 do not clearly reveal the full range of this diversity. This linear plot is dominated by the two strongest materials, dragline silk and viscid silk. The stress–strain curves for tendon collagen and mussel byssal fibres are squeezed together at the bottom of the graph at lower stresses and the stress–strain curves for the rubber-like proteins, elastin and resilin, lie at such small stresses that they can barely be distinguished from the horizontal axis of the graph.

To remove the dominance of the silk curves, the data in figure 1 have been re-plotted on logarithmic axes in figure 2, and this makes it possible to see each individual stress–strain curve clearly. Stress–strain plots on linear axes, such as those in figure 1, can be used directly to determine the stiffness and other mechanical properties listed in table 1, but the distortion of the transformed curves makes their interpretation rather more difficult. However, the figure is useful because it clearly represents the enormous range of properties in this collection of proteins. The upper end of each curve indicates the failure point for each material and this allows us to determine the tensile strength and extensibility for each material. Using these end-points, it can be seen that strength varies by *ca.* 1000-fold and the extensibility by *ca.* 20-fold. Variation in stiffness, as determined by the initial modulus of elasticity (E_{init}), is rather more difficult to extract from the logarithmic plot. For all materials other than collagen there is an initial linear region in the stress–strain curve and this initial slope can be used to estimate E_{init}. The stress achieved when the initial linear portion of each curve is

Table 2. Material properties that can be extracted from the stress–strain curves in figures 1 and 2.

material[a]	modulus, E_{init} (GPa)	strength, σ_{max} (GPa)	extensibility, ε_{max}	toughness (MJ m^{-3})	resilience
elastin (bovine ligament)[1]	0.0011	0.002	1.5	1.6	90%
resilin (dragonfly tendon)[2]	0.002	0.004	1.9	4	92%
collagen (mammalian tendon)[3]	1.2	0.12	0.13	6	90%
mussel byssus, distal (*M. californianus*)[4]	0.87	0.075	1.09	45	28%[5]
mussel byssus, proximal (*M. californianus*)[4]	0.016	0.035	2.0	35	53%[5]
dragline silk (*A. diadematus*)[6]	10	1.1	0.3	160	35%
viscid silk (*A. diadematus*)[6]	0.003	0.5	2.7	150	35%
Kevlar[7]	130	3.6	0.027	50	
carbon fibre[7]	300	4	0.013	25	
high-tensile steel[7]	200	1.5	0.008	6	

[a] References: [1] Aaron & Gosline (1981); [2] Weis-Fogh (1961) and Gosline (1980); [3] Pollock & Shadwick (1994); [4] Bell & Gosline (1996); [5] Waite *et al.* (2002); [6] Denny (1976); Gosline *et al.* (1999); [7] Gordon (1988).

extended to a strain of 1.0 gives the value of the E_{init} and this analysis indicates that stiffness varies by a factor of *ca.* 10 000-fold. Finally, toughness, determined from the area under the stress–strain curve, can only be obtained from linear plots. Values for the modulus, strength, extensibility and toughness given in table 2 are derived from the stress–strain curves in figures 1 and 2.

The data in table 2 give us little insight into the functional significance or design of any of the materials listed. They simply list values for a range of properties that can be documented in mechanical tests. To understand design we must think about the way that elastic devices work in living systems. Indeed, it is important to consider what is meant by the term 'elastic', as this may help us to understand designs that use elastic proteins. Strictly speaking, elastic implies the physical phenomenon of 'elasticity', the subject of Hooke's Law, which states that when a force is applied to an object, that object will deform in proportion to the magnitude of the applied force. Further, when the force is removed the object will return to its original state. Hence, the term elastic means reversible deformation. In addition, reversible deformation implies that the mechanical energy required to deform the object is stored as elastic-strain energy and that all of this stored energy can be recovered in elastic recoil. That is, the elastic efficiency or resilience of a load–unload cycle should be 100%. An alternative meaning for the term elastic is less precise. Elastic is often taken to mean 'stretchy', like a rubber-band, implying that elastic solids can be deformed to large strains with small forces. In the sections that follow we will use these two criteria, reversible elasticity and stretchiness, to develop an understanding of the function, and hence the design, of elastic proteins.

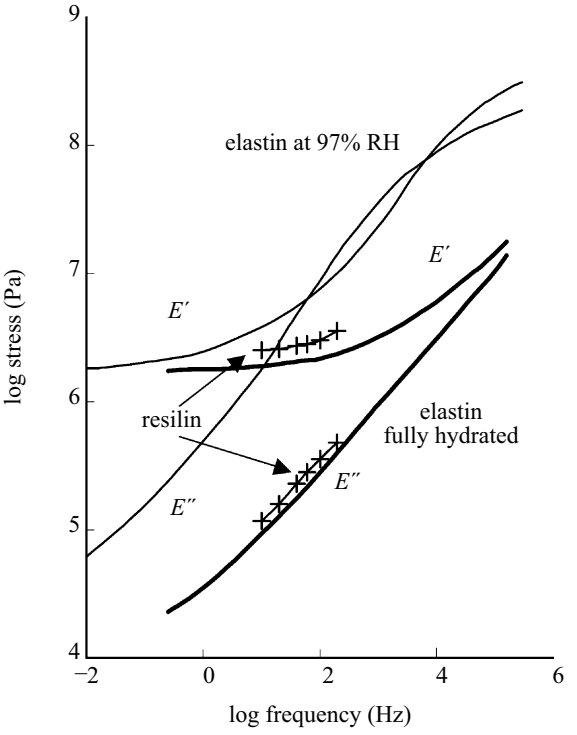

Figure 3. The dynamic mechanical properties of elastin and resilin. The two master curves for elastin were constructed at a reference temperature of 37 °C. Data were replotted from Gosline & French (1979) and Gosline (1980). The data for resilin (crosses) were obtained at room temperature and were redrawn from Gosline (1980). RH, relative humidity.

3. THE FUNCTIONAL DESIGN OF RUBBER-LIKE PROTEINS

The rubber-like proteins, elastin and resilin, are elastic by both criteria described above. Both proteins exhibit reversible deformation with very high resilience. In addition, both proteins are stretchy, reaching maximal extensions in excess of 100%, with a very low modulus of elasticity. This suite of properties implies that a key function of resilin and elastin is to provide low stiffness, high strain and efficient elastic-energy storage components in animal devices. Elastin functions in association with collagen in vertebrate connective tissues where soft, reversible elasticity is required (e.g. in skin and elastic cartilage). In addition, elastin is a major component of arteries, where its stretchiness and ability to store elastic-strain energy allow arteries to smooth the pulsatile flow of blood from the heart, lowering peak blood pressure and the mechanical work of the heart and maintaining a relatively steady flow of blood through tissues. Thus, elastin is definitely an elastic protein, but interestingly it does not behave like one under all conditions.

Figure 3 provides data for the mechanical behaviour of

elastin under a number of circumstances, including varying strain rate, hydration level and temperature. Stretchy materials like elastin achieve their mechanical properties because they contain flexible molecules that can easily change their shape, or conformation, when stretched. The desirable properties of low stiffness, high extensibility and high resilience that are key to elastin's function rely entirely on the ability of the molecules to change their shape faster than the macroscopic shape change imposed by an external force. Thus, these elastic properties are strongly affected by strain rate in a mechanical test. In addition, because conformational change in elastic proteins occurs only in hydrated proteins, elastic properties can also be strongly affected by hydration level. Finally, because conformational changes are driven largely by thermal agitation, properties are also influenced by temperature. The effects of these environmental parameters are illustrated in figure 3, which shows the results of dynamic mechanical tests on mammalian elastin.

Dynamic testing involves the application of small amplitude, sinusoidal deformation to a test sample at a range of test frequencies. Records of time-varying stress and strain are analysed to provide a value for the dynamic modulus, E^*, which is the ratio of the amplitudes of dynamic stress and strain waveforms. In addition, measurement of the phase shift between the stress and strain waveforms allows the dynamic stiffness to be separated into two components. These are: storage modulus, E', which is the stiffness associated with the storage of elastic energy, and loss modulus, E'', which is the stiffness associated with molecular friction and energy dissipation. E' and E'' for elastin are plotted in figure 3 as a function of test frequency. The plots are master curves that were constructed by combining the results of dynamic tests taken at a number of temperatures, using the time–temperature superposition principle for polymeric materials. This process allows one to predict the behaviour of elastin at a reference temperature, in this case 37 °C, over a much broader range of frequencies than can be achieved in laboratory tests (Gosline & French 1979; Gosline 1980; Lillie & Gosline 1990). The logic is that decreasing temperature slows molecular motion, so that mechanical tests carried out at a temperature below the reference temperature will reveal the behaviour at frequencies above the test frequency, and vice versa.

First, consider the bold lines in figure 3, which are labelled elastin, fully hydrated. These curves predict the behaviour of elastin as it exists in living tissues, fully hydrated at 37 °C. Note that at cardiac frequencies (*ca.* 1–3 Hz) E' is approximately two orders of magnitude greater than E''. This indicates a high resilience for elastin at rates of deformation that occur in the cardiovascular system. Resilience, R, can be calculated from these data as follows:

$$R = e^{-2\pi\delta},$$

where δ is the damping factor, equal to the ratio, $\delta = E''/E'$. At 1 Hz, the data in figure 2 indicate a resilience for fully hydrated elastin of *ca.* 90%. However, as the frequency rises above 1 Hz, E' rises slowly, but E'' rises quite rapidly, indicating elastin's entry into the glass transition. When a frequency of 100 Hz is reached, δ has risen markedly to the point where resilience is only 50%. The situation becomes even worse if elastin hydration is reduced, as shown in figure 3 for elastin at 97% relative humidity. This is another master curve with a reference temperature of 37 °C, but the water content is reduced by about 50% (from *ca.* 0.55 to 0.29 g of water per g of elastin). The resulting loss of molecular mobility causes a dramatic change in properties, effectively shifting the master curve to the left by about three decades on the frequency scale relative to the curve for fully hydrated elastin. Efficient elastic behaviour only occurs at frequencies below 10^{-2} Hz. At cardiac frequencies, the resilience is well below 50%. Thus, increasing the frequency or reducing the water content dramatically reduces resilience and this will limit the utility of employing elastin in strain-energy storage devices. This may explain the absence of elastin in the flight system of hummingbirds.

Hovering flight is an energetically expensive process and elastic-energy storage systems can be used to minimize the cost of flight (Weis-Fogh 1972). There is strong circumstantial evidence that hummingbirds function as harmonic oscillators and that they flap their wings at their resonant frequency to reduce the inertial costs of accelerating and decelerating the mass of the rapidly oscillating wings (Wells 1993*a*,*b*; Chai *et al.* 1996). However, even though hummingbirds do have elastin, it is not used in their flight system, probably because its resilience is too low at their wing-beat frequencies (40–70 Hz). Wing elasticity may be provided by the flight muscles or by the tendons connecting these muscles to the wings.

A similar situation is found in the flight systems of insects. There is compelling evidence that insects whose flight is powered by asynchronous flight muscles function as resonant harmonic oscillators (Josephson *et al.* 2000). Insects do not have elastin, but there is another rubber-like protein, resilin, which is found in the wing hinges of some insects (Weis-Fogh 1960). As illustrated in figure 3, the dynamic properties of resilin are quite similar to those of elastin (Jensen & Weis-Fogh 1962; Andersen & Weis-Fogh 1964; Gosline 1980). The curves for E' and E'' run essentially parallel to those for fully hydrated elastin, indicating that resilin, like elastin, is not capable of serving as an efficient elastic-energy storage material for the flight system of insects at high frequencies. Interestingly, resilin's prevalence in insect wing hinges is limited to insects that fly at very modest wing-beat frequencies using synchronous flight muscles. Locusts and dragonflies, for example, have a considerable amount of resilin in their wing systems, but their wing-beat frequencies are at, or below, 25 Hz, where resilin's resilience is more than 70%. Insects with asynchronous flight muscles have wing-beat frequencies in the 100–700 Hz range, and at these frequencies resilin is not a significant component of the wing oscillator. As a consequence, elastic-energy storage must be provided by other sources: the flight muscle and the rigid thoracic cuticle.

It seems that elastin's function as a strain-energy store is restricted to low-frequency load cycles and conditions where elastin can maintain full hydration. It remains for us to quantify its elastic-energy storage capacity under these conditions. To do this, we need information on the durability of elastin in long-term loading to establish the maximum stress level that can be used safely in elastin-based energy storage devices. We have been investigating

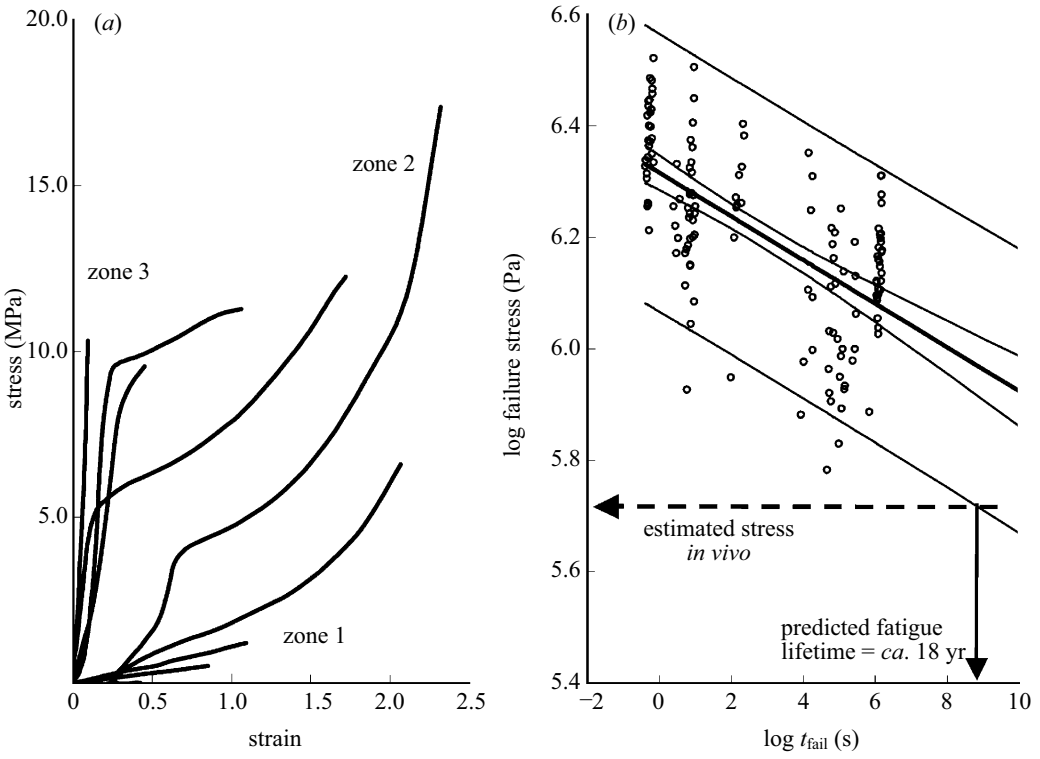

Figure 4. Static-fatigue lifetime estimates for purified, pig-arterial elastin. (*a*) Sample stress–strain curves for constant strain rate tests. Samples tested under full hydration at low strain rates show typical rubber-like behaviour (zone 1), but as the strain rate is increased elastin passes through its glass transition, first becoming tough (zone 2) and then becoming a brittle polymeric glass (zone 3). (*b*) Correlation of failure stress and failure time for fully hydrated elastin at a broad range of strain rates. Extrapolation of this failure envelope to long times provides an estimate of static-fatigue lifetime at *in vivo* stress, as described in the text.

the fatigue lifetime of arterial elastin to assess the possibility that structural fatigue might contribute to its mechanical degradation with age and disease. Elastin is an unusual protein in that it seems not to be replaced during the lifetime of an animal (Shapiro *et al.* 1991; Davis 1993). That is, elastin synthesized during development remains in place through the full life span of the organism, which for humans is 60–80 years. Thus, elastin must be an extremely durable material.

Figure 4 illustrates the results from our experiments to establish the fatigue lifetime of elastin, based on the behaviour of purified pig-arterial elastin. The procedure involves failure tests performed at a wide variety of constant strain rates, which allow us to establish a correlation between failure stress and failure time. The fatigue lifetime at *in vivo* stress is then predicted by finding the failure time associated with that stress level. The problem for elastin is that the fatigue lifetime must be of the order of the lifetime of the animal, which in the case of pig elastin is of the order of 10 years, and it is virtually impossible to conduct fatigue tests of this duration. We have employed the time–temperature shifting procedures described above for the creation of the master curves shown in figure 3 to extend the time-scale of our experiments, and subsequently extrapolated the trend to times that include the full lifetime of animals.

Figure 4*a* shows typical stress–strain tests to failure for arterial-elastin ring samples, tested over an enormous range of effective strain rates. The mechanical properties, including failure stress and failure strain, are strongly influenced by test conditions. At low strain rates, high temperatures and full hydration elastin behaves as a typical rubber, as shown by the low stiffness and high extensibility of the curves in zone 1. Under these test conditions, the failure stresses are low, but the extensions and times to break are high. High strain rates, low temperatures and low hydration give elastin the properties of a rigid polymeric glass, with very high stiffness and low extensibility (zone 3). Failure stresses here are high, but the extensions and times to break are extremely low. At intermediate test conditions, elastin is in the middle of its glass transition, where it is a tough, semi-rigid polymer that fails at high stress and high strain (zone 2).

To assess the fatigue lifetime of elastin in its natural form (figure 4*b*) we have plotted data from 104 samples that showed zone 1-type behaviour. This plot shows the correlation between failure stress and failure time that allows us to estimate durability. The correlation between failure stress and failure time is robust, but the large variation in failure stress limits the accuracy of our predicted fatigue lifetime. In this graph the heavy line is the predicted regression, the inner set of lines is the 95% confidence interval of the regression line and the outer set of lines is the 95% confidence interval for the prediction. It is the lower 95% confidence interval for the prediction that probably establishes the minimum estimate of the fatigue lifetime. We estimate that, *in vivo*, arterial-elastin fibres are stretched by 50% and hence experience a stress

Table 3. The elastic-energy storage capacity of elastin, collagen and spring steel has been calculated from the data given in this table, as described in the text.

material	modulus (GPa)	stress-in-use (MPa)	density ($kg\ m^{-3}$)	resilience	energy storage capacity ($J\ kg^{-1}$)
elastin	0.0011	0.55	1300	0.90	95
collagen	0.12	60	1300	0.90	1000
spring steel	200	600	7800	0.99	115

of *ca*. 0.55 MPa. The dashed, horizontal arrow in figure 4*b* indicates this level. The vertical arrow indicates the predicted fatigue lifetime as *ca*. 18 yr, essentially equal to the lifetime of the animal from which this elastin was isolated. This result provides two important conclusions. First, elastin fibres in life appear to function very close to the upper stress limit allowed by its fatigue lifetime. Second, this stress level provides the information needed to estimate the elastic-energy storage capacity of elastin.

Elastic-energy storage capacity is calculated as follows. The energy per unit volume required to deform elastin to its stress and strain in use, W_{in}, is calculated as,

$$W_{in} = 2(\sigma_{in\ use} \times \varepsilon_{in\ use}),$$

where $\varepsilon_{in\ use}$ is estimated from the ratio of the stress-in-use divided by the modulus of elasticity. The elastic energy recovered in a load cycle, $W_{out} = W_{in} \times R$, where R is the resilience of elastin. Finally, the energy storage capacity can be converted into the units of $J\ kg^{-1}$ by dividing W_{out} by the density of elastin. As indicated in table 3, the elastic-energy storage capacity of elastin is *ca*. $95\ J\ kg^{-1}$. The table also gives the energy storage capacity of spring steel as $115\ J\ kg^{-1}$. It may seem remarkable that a material as soft and as weak as elastin has essentially the same elastic-energy storage capacity as spring steel. This is indeed the case, and it is a good indicator that elastin is a high-quality spring material. Table 3 also gives the spring-energy storage capacity of collagen, which is approximately 10 times greater than that for elastin or steel, and this leads to a completely different class of elastic protein.

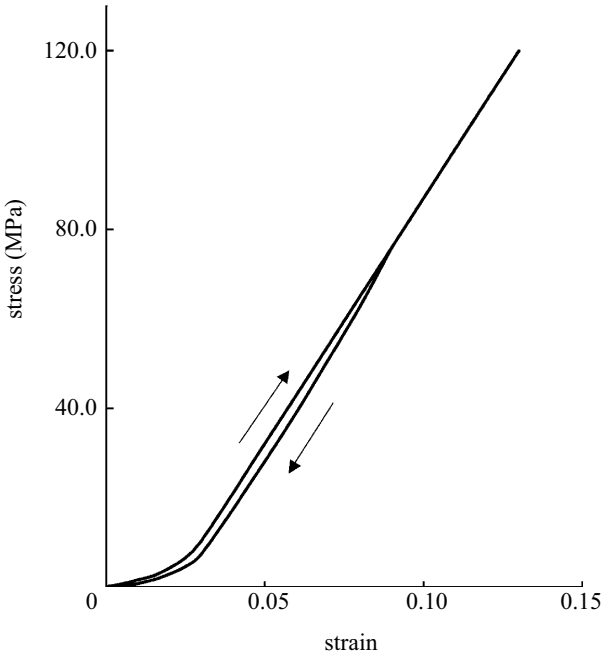

Figure 5. A typical stress–strain curve for tendon collagen, based on data from Ker (1981) and Shadwick (1990). The modulus given is for the linear portion of the stress–strain curve. The load cycle overlying the stress–strain curve indicates that collagen has a resilience of *ca*. 90%. Modulus = 1.2 GPa; strength = 120 MPa.

4. THE FUNCTIONAL DESIGN OF COLLAGEN

Collagen fibres, as seen in tendons, can hardly be described as stretchy, since their extensibility, ε_{max}, is only 0.13. Neither is collagen soft, since its modulus is approximately 1000 times greater than that of elastin or resilin. It is also much stronger and somewhat tougher than elastin or resilin (see table 2). So, why consider collagen to be an elastic protein? Figure 5 shows a typical stress–strain curve to failure for tendon collagen, with a load cycle to about 50% of breaking stress superimposed upon it. Note that tendon, and virtually all other collagen-containing connective tissues, have a 'J-shaped' stress–strain curve. At low strains the slope is low, but as extension proceeds it rises gradually and becomes constant when the collagen fibres become aligned and then finally stretched. These data, and particularly the load cycle, show that collagen is definitely capable of reversible deformation and it is this aspect of collagen that establishes it as an elastic protein. Indeed, the resilience of tendon collagen is *ca*. 90% (Ker 1981; Shadwick 1990). In spite of this, collagen does not always function as an elastic protein in animals. Rather, collagen is frequently arranged in parallel with elastin fibres to form connective tissues, and in these circumstances the 'stretchy' elasticity of the tissue is due primarily to the elastin. The collagen provides a network of wavy, reinforcing fibres that become aligned in the direction of stretch. When taut, this network limits tissue deformation and prevents the rupture of the softer and weaker elastin fibres. Some tendons, however, do function as elastic devices and have a remarkable energy storage capacity.

Ker *et al*. (1988) found that there are two distinct classes of tendons, based on the stresses these tendons experience *in vivo*. Some tendons seem to be over-strong, with safety factors between 8 and 15. In this case, safety factor is defined as the tendon's breaking force divided by the maximum isometric force generated by its muscle. Other tendons, particularly those found in the ankle and digital extensor muscles in the limbs of cursorial animals, have much lower safety factors, typically between 1 and 2.

Referring to the stress–strain curve in figure 5, we can see the functional consequences of these differences. The stress–strain curve in this figure is typical of both the

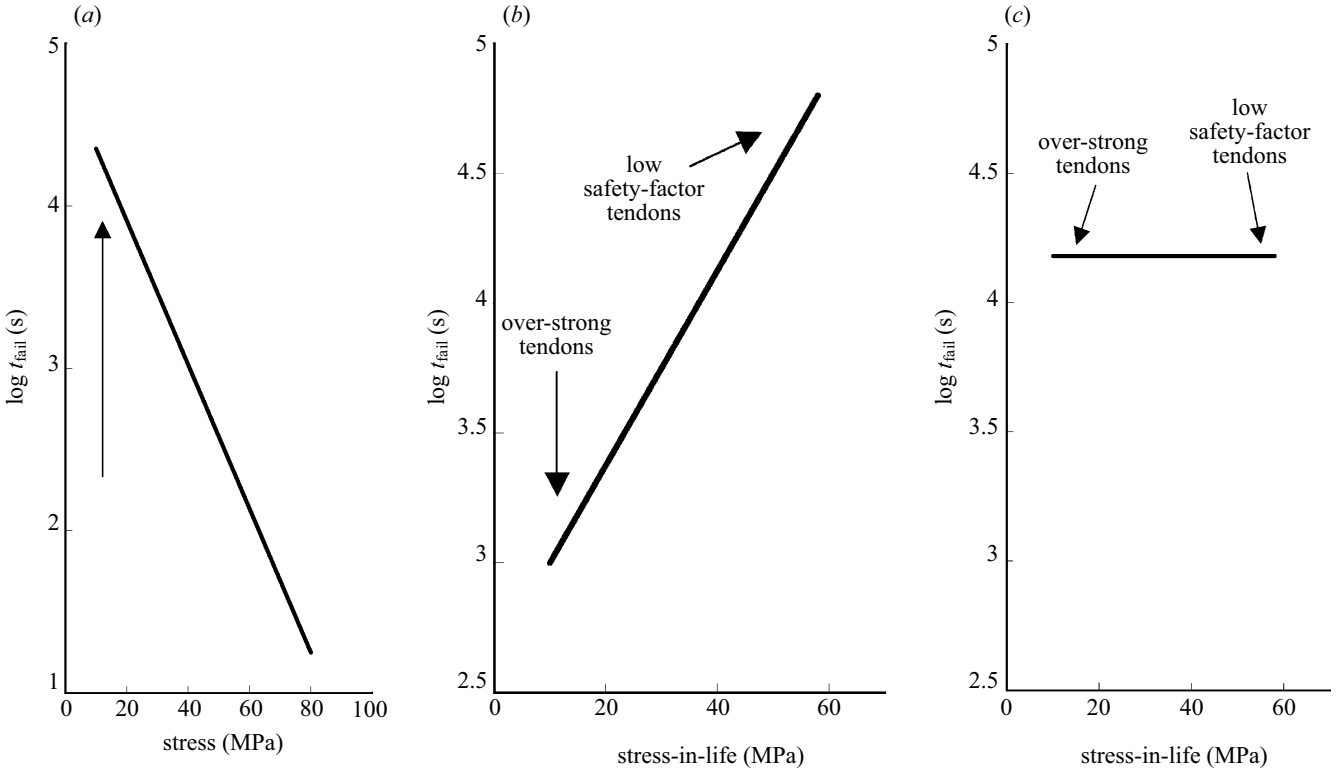

Figure 6. The fatigue lifetime of tendon collagen. (*a*) The static-fatigue lifetime (t_{fail}) of wallaby tail tendon at a range of test stress levels. The vertical arrow indicates the maximal stress-in-life for this tendon (12 MPa). (*b*) The static-fatigue lifetime (t_{fail}) obtained for nine different types of wallaby tendons, all taken at a test stress of 50 MPa, are plotted at the stress-in-life for each tendon. (*c*) The static-fatigue lifetime (t_{fail}) for the nine wallaby tendons, obtained at the stress-in-life for each tendon, are plotted against the stress-in-life. The lines plotted here are from regressions in figs 2, 4 and 5 of Ker *et al.* (2000). Specific information on the identity and properties of the different tendons can be found in that paper.

over-strong and the low safety-factor tendons. Both classes of tendon have essentially identical stiffness in the linear zone and tensile strength (Pollock & Shadwick 1994) and this means that the strain levels seen in life by these two tendon types must be very different. In the over-strong tendons, tendon strain should never exceed 0.02 (i.e. 2% extension), even when the attached muscles are maximally activated. Under sub-maximal loading, the strain in these tendons will be even smaller. Functionally, the over-strong tendons provide rigid links between muscle and bone. On the other hand, during locomotion, the low safety-factor tendons experience much larger strains in their normal function, strains that approach the failure strain. This exceptional difference in strain level is a direct reflection of the design of low safety-factor tendons to function as springs that conserve energy in running locomotion (Alexander 1988). Indeed, these low safety-factor tendons could be called spring tendons and they are probably the pre-eminent strain-energy storage devices in animal skeletons.

The elastic-energy storage capacity of tendon collagen can be calculated from the data in figure 5, with some additional information on the fatigue behaviour of tendon collagen. These data are presented in figure 6. Figure 6*a* shows the static fatigue behaviour of wallaby tail tendon, an over-strong tendon with a safety factor of approximately 10. The log of fatigue lifetime, in seconds, is plotted as a function of static stress level, and the regression line shown was based on over 90 fatigue tests. There is a strong correlation between stress and fatigue lifetime, with fatigue lifetimes ranging from *ca.* 15 s at the highest stresses to over 10^4 s at low stress. The tail tendon sees a 'stress-in-life' of 12 MPa, as determined from the maximal isometric force of the tail muscle. The vertical arrow indicates the intersection of the stress-in-life with the regression line, and this intersection point indicates that the fatigue lifetime of wallaby tail tendon at its stress-in-life is *ca.* 2×10^4 s, or 5.5 h. It is interesting that the static-fatigue lifetime of tendon collagen is many orders of magnitude shorter than that of arterial elastin, but perhaps this is because the stress levels experienced by tendons are so much higher. It certainly confirms that tendons must remodel and repair themselves throughout the life of an animal.

Figure 6*b* shows the regression line obtained when the fatigue lifetime for nine different wallaby tendons, all determined at a fixed test stress of 50 MPa, is plotted as a function of the stress-in-life for each type of tendon. The range of tendons used includes several that are over-strong and have low stress-in-life, such as the tail tendon and digital flexor tendon. At the other extreme are limb extensor tendons, the plantaris and digital extensor tendons, which are low safety-factor tendons and have a high stress-in-life. Interestingly, there seems to be a large variation in fatigue lifetimes that correlates with the stress-in-life seen by each type of tendon. This correlation indicates that those tendons that have evolved to function at high stress (the spring tendons) have also developed a better fatigue

resistance than the over-strong, link tendons. Indeed, it suggests that if the fatigue lifetime for all tendons is measured at the stress-in-life, then all tendons may have very similar fatigue lifetimes. Figure 6*c* shows that this is indeed the case. All tendons have essentially the same fatigue lifetime, *ca*. 5 h at the stress-in-life.

With this information, we can estimate the elastic-energy storage capacity of the collagen in spring tendons. We will assume that spring tendons evolved to function at stresses up to their stress-in-life, and thus that these tendons can be loaded to 60 MPa repeatedly in energy-storing load cycles. Elastic-energy storage capacity was calculated as described above for elastin, and the values are shown in table 3. As noted above, collagen has an elastic-energy storage capacity that is approximately 10 times greater than that of elastin or spring steel. It is important to note that this storage capacity is not the highest possible, as the limb extensor tendons in animals larger than the wallaby experience considerably higher maximal stress-in-life. In reality, the upper limit of elastic-energy storage capacity in collagen is probably closer to 2000 J kg^{-1}.

Finally, the spring-energy storage potential for collagen needs to be considered in light of the other properties that have been determined for collagen. Collagen does function in circumstances where its strength and toughness are crucial but, as listed in table 2, collagen is not particularly stiff, strong or tough when compared with other materials. For example, the spider silks are 3–8 times stronger and 20 times tougher than tendon collagen, and high-performance man-made fibres can be stronger still. Thus, collagen can be described as an exceptional design for elastic-energy storage and a modest design for strength and toughness. To find elastic proteins with exceptional design for strength and toughness, we will have to look at the remaining elastic proteins in our list, mussel byssal fibres and spider silks.

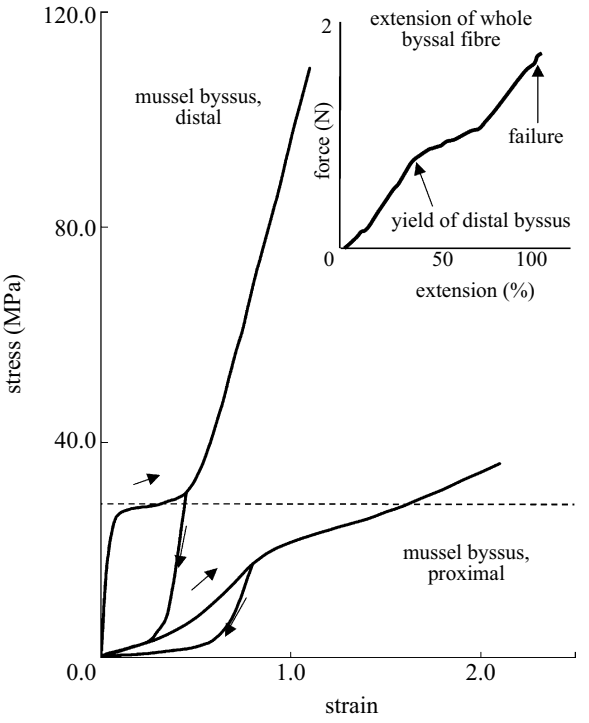

Figure 7. The mechanical properties of mussel byssal fibres. The stress–strain plot shows the material properties of proximal and distal portions of byssal fibres from the mussel, *M. californianus*. The dashed line indicates that the yield level for the distal fibre occurs below the failure stress for the proximal fibre. The inset graph shows typical force–elongation for a whole byssus fibre. Data have been replotted from Bell & Gosline (1996).

5. THE FUNCTIONAL DESIGN OF MUSSEL BYSSAL FIBRES

The fibres of the mussel byssus are used to attach the animal to rocks in the wave-swept marine intertidal zone. The fibres look like good candidates for elastic proteins by the 'stretchy' criterion, as demonstrated by the force–extension curve for whole byssus fibre in figure 7. Whole byssal fibres stretch by about 100% before breaking, but the origins of the whole-fibre behaviour are complex because the fibres are composed of two segments, a distal segment that makes up about 80% of the fibre and a short, proximal segment that makes up the remainder of the fibre. The material properties of these two segments from *Mytilus californianus* are shown as stress–strain curves in figure 7 (Bell & Gosline 1996). The proximal segment is extremely stretchy, with an extensibility of *ca*. 2.0, suggesting a rubber-like behaviour, but its stiffness and strength are about an order of magnitude greater than those for elastin and resilin (table 2). The distal region is somewhat less extensible, with an ε_{max} of *ca*. 1.0, but its stiffness and strength approach those of collagen. Because of the combination of high strength and extensibility, the toughness of both proximal and distal byssal threads is approximately an order of magnitude greater than that of resilin or elastin and about six times greater than that of tendon collagen. The toughness of byssal fibres is quite comparable with that of Kevlar and carbon fibres (table 2), and this high toughness is certainly key to the survival of mussels in the marine intertidal zone.

Interestingly, byssal fibres would not be considered as elastic materials if judged by the criterion of reversible elasticity. First, the large, open load cycles of the stress–strain curves in figure 7 indicate a low resilience. Second, it is not clear if the deformation of these fibres is fully reversible, in that the fibres may not immediately return to their original dimensions following load cycles to high, but sub-maximal strain levels. The resilience values in table 2 are for the first-cycle behaviour of fibres from *Mytilus galloprovincialis*, taken from Waite *et al*. (2002) in this issue. Subsequent load cycles show increased resilience, so the mechanical properties are quite complex, but it is clear that these fibres do not function as efficient springs (Waite *et al*. 2002). Rather, their strength and toughness, properties that allow mussels to resist dislodgment by breaking waves or hungry starfish, are probably enhanced by their low resilience.

Polymeric materials that dissipate mechanical energy through molecular friction, and hence have low resilience, usually have enhanced toughness. The correlation between low resilience and high toughness in polymeric materials can clearly be seen for elastin in figure 4*a*. In fully hydrated elastin, where resilience is high (zone 1), stiffness, strength and toughness are low (table 2). As the

water content and temperature are lowered and the strain rate increased, elastin enters its glass transition (zone 2). Here it exhibits higher stiffness and extensibility but lower resilience, leading to a toughness that is at least an order of magnitude higher. It is worth noting that because elastin must be dehydrated or cooled to exhibit zone 2 behaviour at moderate strain rates, it may never function in this region of its viscoelastic curve under natural conditions. However, mussel byssal fibres achieve similar toughness levels when fully hydrated in seawater, so it may be fruitful to discover the molecular origins of this enhanced toughness.

Our analysis so far indicates that byssal fibres are exceptional for their stretchiness and for their toughness. It is not clear, however, if these properties alone are key to the function of the byssus apparatus. It is possible that the yield behaviour, seen particularly clearly in the distal byssus, might also play a role in its function. Mussels build their byssus apparatus to anchor themselves to rocks in the intertidal zone, where the waves can strike them from a variety of directions. As a consequence, they create a multidirectional array of fibres, with only a fraction of the fibres orientated to resist forces coming from any particular direction. However, as these fibres are elongated by a breaking wave, other fibres reorientate and are recruited into the load-bearing set of fibres. Clearly the stretchiness of the fibres facilitates this process, but in addition, it seems that a significant yield in their force-deformation behaviour has the same effect (Bell & Gosline 1996). Unfortunately, we know little about the stress-in-life or the fatigue behaviour of these fibres, and it is clear that more research in needed before we truly understand the design of these interesting elastic proteins.

6. THE FUNCTIONAL DESIGN OF SPIDER SILKS

Spiders produce a variety of structural polymers, called silks, which have evolved to function in air rather than in aqueous media and whose mechanical properties range from rubber-like to extremely rigid. Given this range of properties, it is not possible to categorize all silks with a single set of criteria. We will therefore focus on the two silks that are best studied and whose properties probably span the range of properties that exist in spider silks. These are the viscid silk, which forms the glue-covered catching spiral of the orb web, and dragline silk, the rigid silk used as a safety line and for the frame of the orb web. Stress–strain curves to failure for these two silks, with load cycles overlaid, are shown in figure 8.

Viscid silk easily meets the 'stretchy' criterion for an elastic protein because, like elastin and resilin, it has a low initial stiffness, $E_{\text{init}} = 3$ MPa, and high extensibility, with $\varepsilon_{\text{max}} = 2.5$. However, it is a great deal stronger than elastin or resilin and its tensile strength of 450 MPa makes it the strongest rubber-like material known. The rubber-like behaviour of viscid silk is unexpected for an elastic protein because viscid silk fibres function in air and other rubber-like elastic proteins are brittle polymeric glasses when air-dried. The difference for spider's viscid silk is that molecular mobility in its protein network is maintained by the presence of molar concentrations of low molecular weight organic compounds in the glue (Vollrath *et al.* 1990). These glue compounds are hygroscopic and draw water out of air (Townley *et al.* 1991). Some of the glue compounds penetrate the silk network where they, along with their associated water molecules, plasticize the silk proteins and maintain molecular mobility. In addition, water absorbed into the glue layer keeps the glue sticky for its role in prey capture. If viscid silk is dried over P_2O_5 to remove all water (Vollrath & Edmonds 1989), or if the glue is washed off and the fibres allowed to air dry at ambient humidity (Gosline *et al.* 1995), the fibres become rigid, as expected for an un-plasticized protein polymer. As rubber-like elasticity in viscid silk is maintained by complex mechanisms, long-range elasticity is probably an important property for the silk's function in the web.

Dragline silk, in contrast, is not very stretchy. Its initial modulus at 10 GPa and tensile strength at 1.1 GPa make it more like a rigid super-fibre such as Kevlar. This behaviour is a direct reflection of the air-dried, non-plasticized condition of the proteins in these fibres. The extensibility of dragline is about 0.3, and although this is about an order of magnitude greater than that of Kevlar or carbon fibre (see table 2), it is less stretchy than most elastic proteins. It is not clear, therefore, whether the label of elastic protein is appropriate for dragline silk. Perhaps the criterion of reversible elasticity will clarify the issue.

The load cycles in figure 8 show typical data for viscid and dragline silks from the spider *Araneus diadematus*. Denny (1976) studied both the viscid and dragline silks of *Araneus sericatus* and observed that both silks failed to return to their initial dimensions at the end of an initial load cycle, but that subsequent cycles had consistent, reversible behaviour. Interestingly, the fibres did return to their initial length and showed the first-cycle behaviour if left slack for about 10 min. Characterization of the shape of the load cycles reveals that both viscid and dragline silks have very low resilience, with a first-cycle resilience of 30–40% for both materials. Shao & Vollrath (1999) obtained similar results in load-cycle tests on dragline silk from *A. diadematus* and from *Nephila edulis*; the silks failed to return to their initial length and had very low resilience.

These properties obviously make the web silks poor candidates for use in elastic-energy storage devices. But this makes sense, since the orb web functions as an energy-dissipating device. As shown in figure 8, a flying insect hitting one of the stretchy viscid silk strands will deflect the strand as it absorbs the kinetic energy of the insect. If the energy of this impact is stored as elastic-strain energy, then elastic recoil of the silk would probably catapult the insect back out of the web. To minimize recoil, absorbed energy must be dissipated as heat through molecular friction. The situation for dragline silk is similar. Its function in the orb web is to support the catching spiral formed by the viscid silk and its ability to dissipate the energy of impact is equally important to the prey capture process. In addition, its function as a safety line is to absorb and dissipate the energy of a falling spider. Thus, a key function for both dragline and viscid silks is to dissipate energy in impact loading, which can be achieved because polymers with low resilience have enhanced toughness. The values for toughness in table 2 indicate that these silks are tougher by a factor of 3–4 than the other materials listed, including the high-tech super-fibres. This, of course, is the key property for the web silks. They are energy-absorbing devices that function only once in the capture of a prey

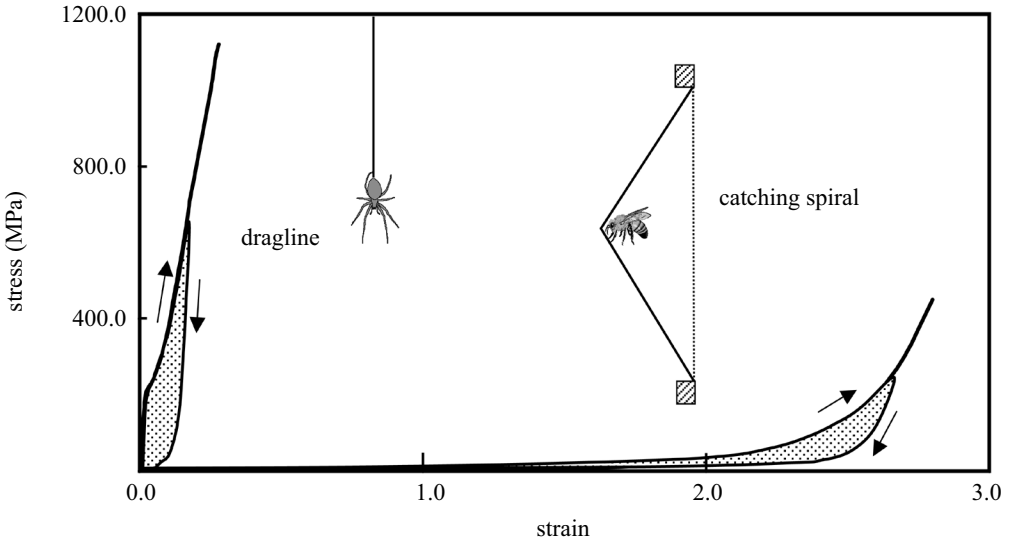

Figure 8. Stress–strain curves and overlaid stress–strain cycles for dragline and viscid silks from the spider *A. diadematus*, after Gosline *et al.* (1999).

item or in the fall of a spider and because of this they are essentially disposable items for which concepts like fatigue are largely irrelevant.

However, the mechanical environment in which they function may strongly affect their properties. Figures 3 and 4 illustrate how strain rate, water content and temperature strongly affect the mechanical behaviour of elastin. These parameters will also have a strong effect on the behaviour of silks. Dragline silk shows dramatic changes in strength and toughness when the strain rate is increased to mimic those occurring in prey capture. When dragline is immersed in water it swells and is transformed into a rubber-like elastic material. These two transformations are illustrated in figures 9 and 10.

Figure 9 shows the effect of increasing strain rate from a low value of 0.005 s^{-1} to *ca.* 30 s^{-1}, a shift that decreases the time to failure from *ca.* 60 s to *ca.* 10^{-2} s. The high strain-rate impact tests were designed to mimic an insect flying into a silk strand in a web and the changes in mechanical properties are impressive (Gosline *et al.* 1999). There is a large increase in initial modulus, rising from *ca.* 10 MPa to greater than 30 MPa, and there is a similar rise in tensile strength. Most test samples fail at stresses of *ca.* 2 GPa, but in some instances, as shown by the two extreme curves in figure 9, strength values in the range of 3–4 GPa are seen. That is, strength may approach that of the polymeric super-fibres, but the silk's extensibility remains high. This combination brings the toughness to exceptionally high levels. Currently, we do not know the maximum toughness possible nor the conditions needed to achieve the maximum, but we estimate that the toughness of spider dragline may rise to 500–1000 MJ m^{-3}. This would make spider dragline silk the toughest material known to man, and there are obvious benefits to the spider of having a super-tough material for the manufacture of its orb webs. We do not have data for the impact loading of viscid silk, but it is probable that high strain rates will increase its toughness as well.

Figure 10 shows the effect of immersing dragline silk in water. Figure 10*a* shows a force–elongation curve for the

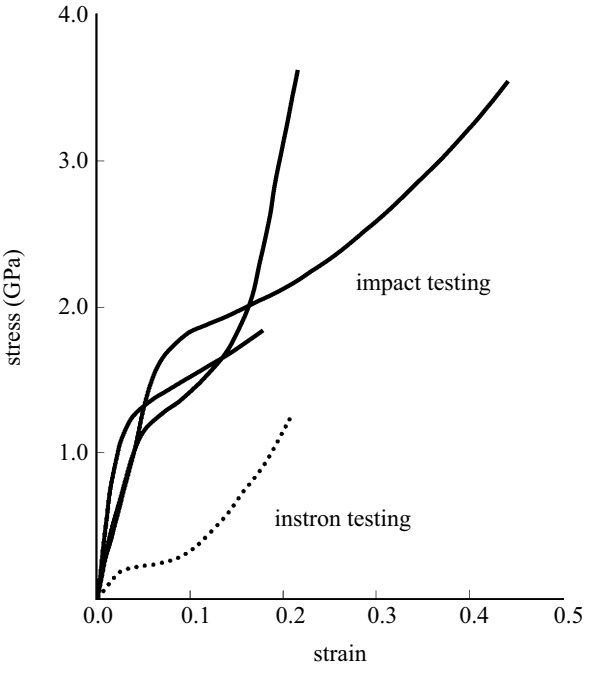

Figure 9. The effect of strain rate on the mechanical properties of dragline silk from *A. diadematus*. Data have been re-plotted from Gosline *et al.* (1999). Impact testing strain rate = 30 s^{-1}; instron testing strain rate = *ca.* 0.005 s^{-1}.

dry dragline (dashed line), starting from a reference length of 1.0, rising to failure at a length of *ca.* 1.3. When dragline is immersed in water (solid line) the silk absorbs water, roughly doubling its volume, and contracts to about half of its dry length. When the hydrated, contracted dragline is extended, it exhibits rubber-like mechanical properties (Gosline *et al.* 1984). Figure 10*b* shows stress–strain curves for several samples of hydrated, contracted dragline from *A. diadematus*, showing that its initial stiffness is *ca.* 10 MPa, or roughly an order of magnitude greater than that of elastin or resilin. In its rubber-like state, *Araneus*

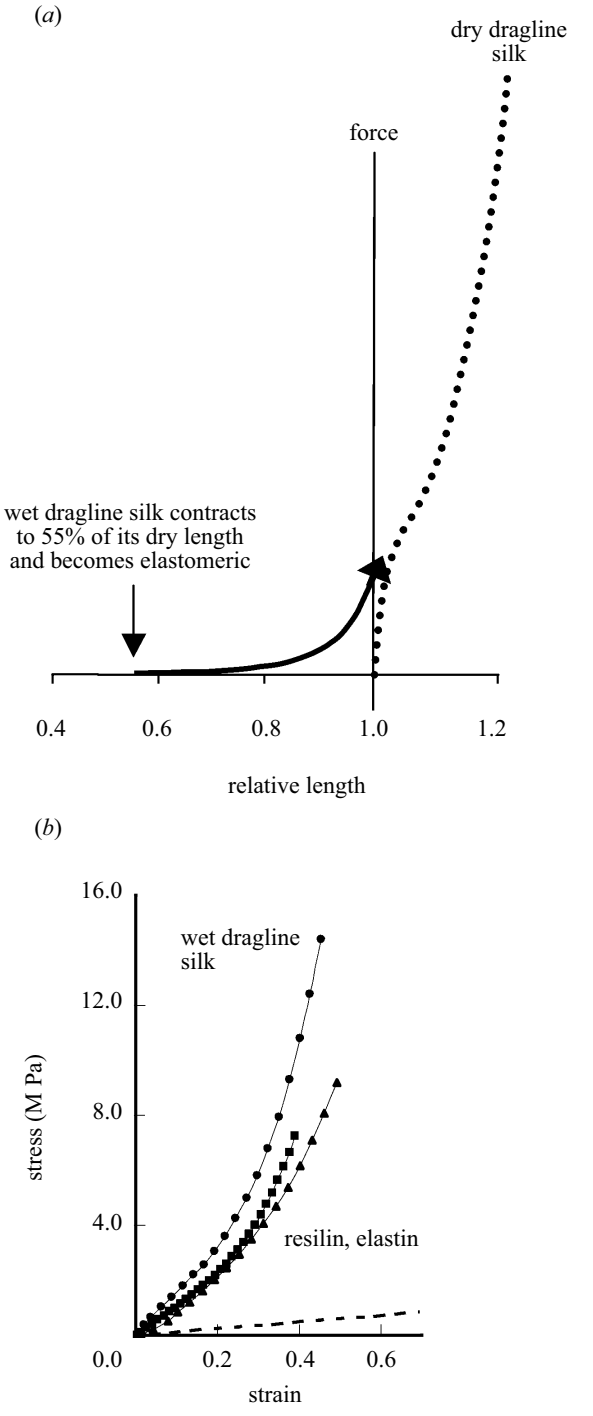

Figure 10. The effect of water on the mechanical properties of dragline silk. (*a*) The change in the force–elongation behaviour of dragline silk that occurs when it is immersed in water. (*b*) The stress–strain curves compare the material properties of wet dragline silk with the rubber-like proteins elastin and resilin. The initial stiffness for wet dragline silk is *ca.* 10 MPa.

dragline has an extensibility of *ca.* 1.3 and a strength similar to that of viscid silk (Shao & Vollrath 1999), making it, like the viscid silk, a truly exceptional elastomer. Thus, dragline silk is an elastic protein, but it exhibits its elasticity only when fully hydrated. It is therefore the one example of an elastic protein whose primary function does not arise from its elasticity.

7. CONCLUSIONS

The structural proteins described in this paper are extremely diverse in their material properties. Under appropriate conditions, all exhibit reversible elasticity and/or stretchiness and can all therefore be classified as elastic proteins. When we consider the functional roles that these proteins play in the lives of the animals, we find that the mechanical properties crucial to their function usually, but not always, encompass some aspect of their elasticity. Not all of the elastic proteins are exceptional in their material properties, but all represent good designs because the properties that define their function are well matched to the environmental conditions. Elastin and resilin work well as strain-energy storage devices at low frequencies and long times, but they have material properties that could probably be equalled by commonly available synthetic materials. Collagen fibres and spider silks, on the other hand, are at the material limits for their respective classes of polymeric materials and they are truly exceptional materials. Collagen has unmatched capacity for the storage of elastic-strain energy and spider silks have unmatched toughness. In addition, when we test the natural elastic proteins under environmental conditions beyond those seen in their normal function, we discover a much broader range of material properties. It is this full range of properties that is available for exploitation through biotechnology. For example, dragline silk becomes rubber-like when hydrated, but it retains essentially all of its remarkable strength, making it a candidate as a high-performance elastomeric material for bioimplantation. Mussel byssal fibres normally function in seawater, but when dry, or perhaps when plasticized by other solvents, they may achieve greater strength with little compromise in extensibility. Clearly, there are interesting and useful molecular mechanisms in elastic proteins that could be incorporated into the design of novel man-made materials.

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