Rheological properties of the extensible intersegmental membrane of the adult female locust

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The intersegmental membrane of the mature female locust displays remarkable long range elasticity (it can stretch elastically up to a strain of 15) and a remarkably low modulus. It also has a large viscous component. These properties are probably due to the protein fraction. Rheological tests suggest that the basis of the elasticity of the membrane is not a rubbery one, but a close-packed system of loosely bonded chains in a moderately specific conformation. Such a basis of elasticity may hold for other low-modulus proteins.

1. Introduction

The adult female locust digs a hole 8 to 9 cm deep in sand in which to lay her eggs. The normal length of her abdomen is about 2.5 cm. Part of the increase in length is achieved by unfolding the telescoped intersegmental membranes, the remainder by stretching the membranes (Fig. 1).

The force required for stretching the membranes comes solely from the ovipositor valves at the tip of the abdomen, which form a functional digging unit together with the last two
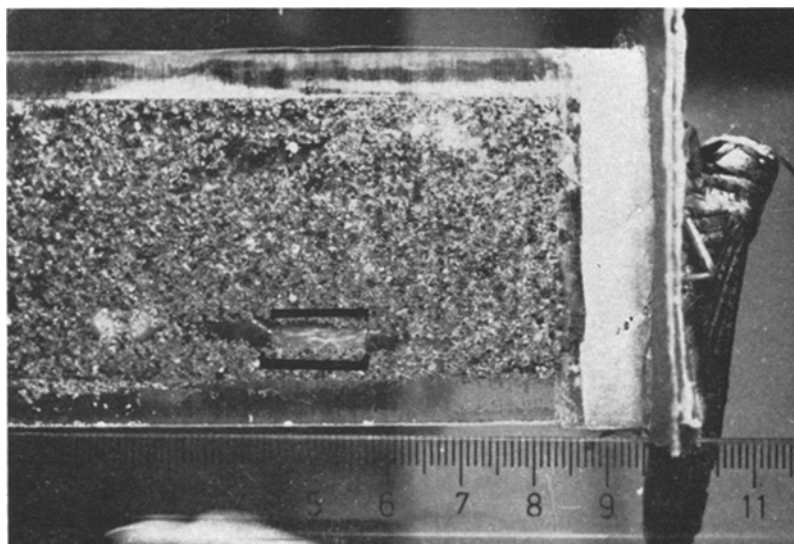

Figure 1 A female locust laying her eggs in sand in a "Perspex" observation chamber. One of the three extensible intersegmental membranes is visible, and is marked with square brackets. The scale is in cm and mm. (Figure turned through 90°.)

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segments of the abdomen [1]. Because the insect does not hold on to the sides of the hole with her ovipositors all the time, it is necessary that the membranes have a large viscous component. This paper reports one aspect of the visco-elasticity of the membranes.

The morphology of the membranes is complex [2], but in simple terms, the two main components are chitin and protein. Most of the chitin is orientated normal to the direction of extension in the living animal: chitin is inextensible. The protein is not cross-linked [3], and is assumed to be responsible for most of the observed mechanical properties of the material.

Our aim in this investigation was to examine the rheological properties of the membrane.

2. Materials and methods

2.1. Sample preparation

A sample for testing was prepared by dissecting the extensible membranes from a number of locusts, and allowing them to imbibe distilled water. This does not affect the linear mechanical properties of the cuticle [3], but (a) facilitates the removal of the inextensible folded epicuticle [2]; (b) increases the volume of the sample; (c) makes the individual pieces of cuticle adhere to each other much better, giving the sample the appearance of sputum; and (d) the increase in volume probably allows greater separation of the chitin microfibrils, making their presumably random orientation in the rheogoniometer of less importance in the measured parameters. This water solvates the cuticle but does not dissolve it in any way.

2.2. Instrument and modifications

The instrument used for measuring the rheological properties was a Weissenberg Rheogoniometer (Sangamo Controls, Ltd) modified for the present purpose.

The total volume of sample available, even after pooling the material obtained from as many as twenty-five insects, was only about 1 ml. The rheogoniometer is capable of making measurements on such a small sample only if the smallest platens are used. However, with these platens, the sensitivity is at its lowest, and since the locust membrane is a very soft material, it is essential that the sensitivity be as high as possible. A greater sensitivity was achieved by replacing the cone and plate system of the standard instrument by a system of concentric cylinders. For the same volume of sample and

same shear rate, this gave an improvement in sensitivity by a factor of 6. The outer cylinder, or cup, was machined from aluminium and had an internal diameter of 10.8 mm. The inner cylinder, also machined from aluminium, was 9.75 mm in diameter and 12 mm long. A cylindrical trough, 75 mm in diameter and 30 mm deep, was machined integral with the cup. This trough served as a water bath and provided easier and more effective temperature control of the sample than the standard unit. The increase in sensitivity and the easier thermostatting with this arrangement were felt to outweigh the slight loss of rigour incurred in evaluating the results, compared with the cone and plate system. A further increase in sensitivity was obtained by replacing the standard torsion bar by one made from beryllium-copper 0.38 mm thick, but otherwise having the same dimensions as the standard one. With this enhanced sensitivity, the instrument was susceptible to draughts within the laboratory, and these were excluded by enclosing the whole of the rheogoniometer test head in a "Perspex" box.

2.3. Theory and calibration

In the theory and calibration of the instrument, several factors must be taken into account. The rate of shear across the gap between concentric cylinders is not constant. Furthermore, when the material filling the gap is non-Newtonian, the velocity profile is a function of the properties of the material, and hence, in a material under investigation, is unknown. However, provided that the gap is small compared with the radii of the cylinders, a mean value for the shear may be used with little loss of accuracy, even for substantial departures from Newtonian behaviour [4]. For the present purposes the mean value was taken as [5]

$$\bar{\gamma} = \omega \, \frac{2ab}{a^2 - b^2}$$

where $\bar{\gamma}$ = mean shear rate, ω = angular frequency, a = inner diameter of cup, b = outer diameter of inner cylinder. A similar argument applies to the relation between torque applied to the cylinders and the rate of revolution. Only that torque associated with the flow of the material in the annular space may be calculated accurately. The drag due to the material in the end spaces above and below the inner cylinder also makes an appreciable contribution which cannot readily be calculated. The instrument was

therefore calibrated using standard (Newtonian) oils whose viscosity had been determined accurately in an Ostwald viscometer.

2.4. Experimental measurements

The sample, after being prepared as described above, was quick-frozen and stored at a temperature below $-20°C$ for a few days, until required. It was then allowed to warm up to room-temperature and introduced into the cup of the rheogoniometer. The water bath was filled with water at the required experimental temperature: this temperature was easily maintained constant to $\pm 0.1°C$ by occasional addition of a few drops of warmer or colder water as required.

The measurements were all made on the same sample. At first, the oscillatory mode of operation was used at angular frequencies between 0.063 and 1.57 rad. sec^{-1}, and within the temperature range 2 to $40°C$. A few observations were also made on the effect of direct shear and subsequent recovery at $20°C$. In these, the sample was sheared at a constant rate of 0.24 sec^{-1}. This was applied for approximately $3\frac{3}{4}$, $7\frac{1}{2}$ and 15 sec, corresponding to total applied strains of 0.9, 1.8 and 3.6. At the end of the period of shearing, the drive was stopped and the stress which had built up was allowed to relax until the rate of relaxation had become slow (about 0.5 min). At this point the drive was applied in the reverse direction for the same period as in the forward and the stress was then allowed to relax once more.

3. Results

3.1. Oscillatory shear

A modulus, G, was calculated from the measured amplitude of the stress and the calculated amplitude of the strain when the rheogoniometer was operated in the oscillatory mode. Making the assumption that the material possesses linear viscoelasticity, this modulus was resolved into two components: an elastic opponent, G', in phase with the applied strain and G'', a viscous component in quadrature with the applied strain. These were calculated as $G' = G \cos \delta$ and $G'' = G \sin \delta$, where δ is the phase angle, which was read from the recorder charts as the phase lag of the strain at the point of zero stress. The dynamic viscosity was calculated by dividing the loss modulus, G'', by the angular frequency ω.

Applying the principle of time-temperature superposition [6], the measurements made at the

several temperatures were converted to a *standard temperature*, $(T_g + 50)$ °C, i.e. with T_g (the glass transition temperature, determined in linear tests on the material [3]) $= -17°C$, the standard temperature was $33°C$. Since the greater bulk of the sample was water, the correction for density at the different temperatures (ρ_0/ρ) was taken as that for water, and approximated as unity. The shift factor, a_T [8], is essentially empirical, and appears to hold as a temperature correction for ω for a wide range of rubbery polymers. The values of a_T used in this study are those for a rubbery polymer, despite the conclusions we draw that locust intersegmental membrane elastomer is not rubbery. The inference that a_T is appropriate for non-rubbery long-chain molecule elastomers is itself a point of interest. In this way the variation of the modulus was effectively extended to cover some six decades of shear rate.

The shape of the response curve is shown in Fig. 2, in which the variation of stress is shown
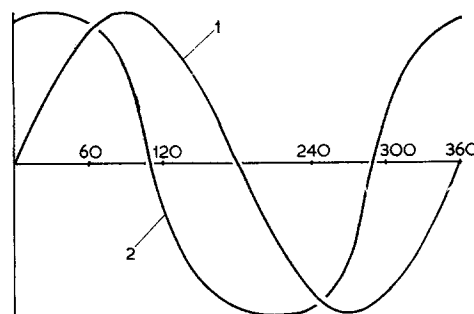

Figure 2 Applied strain (curve 1) and stress developed within the material (curve 2) during one cycle of deformation.

for a sinusoidal variation of imposed shear. This type of response curve was obtained at all frequencies and at all temperatures used, and must be regarded as characteristic of the material. The implication of this type of response, in terms of the viscoelasticity of the sample, is that either the modulus, or the phase angle, varies somewhat during the cycle with a frequency of twice that of the imposed shear. It is because of this that it was necessary to define the modulus and the phase to be used in the subsequent calculations of the elastic and viscous components in terms of fixed reference points during the cycle.

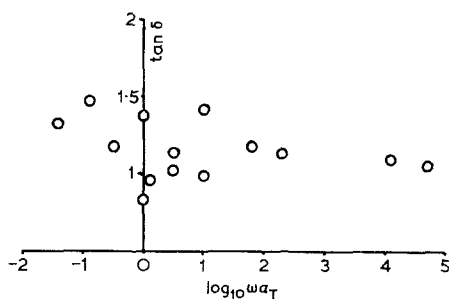The phase angle, δ, could not be read from the

Figure 3 Variation of tan δ with reduced frequency ωa_T.

charts with great precision, but was estimated to the nearest degree. Over the whole strain range it varied by only a few degrees and was always close to $50°$. Both temperature and frequency had a small effect. The transformation of temperature to time, results in the variation seen in Fig. 3, which shows that tan δ remains slightly greater than one over a frequency range of six decades.

The elastic component of the modulus, G' shows a small increase with frequency and a decrease with increasing temperature. When these were combined, the resulting points, plotted in Fig. 4, show once again only small changes over
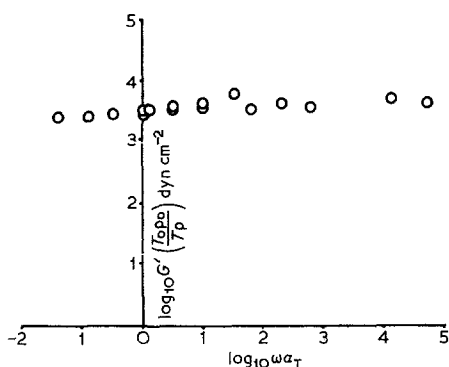

Figure 4 Variation of the elastic component of the modulus, G', with reduced frequency, ωa_T.

the six decades of frequency. The points, shown in Fig. 5 for G'', the viscous component, show a similar pattern.

If the dynamic viscosity is plotted instead of the loss modulus, the curve of Fig. 6 is obtained. With the exception of the two points on the extreme left, which were calculated from the data at $40°C$, the curve is a remarkably good straight line with a slope of almost -1, over more than five decades.
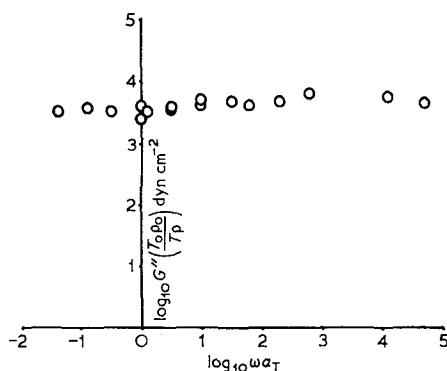

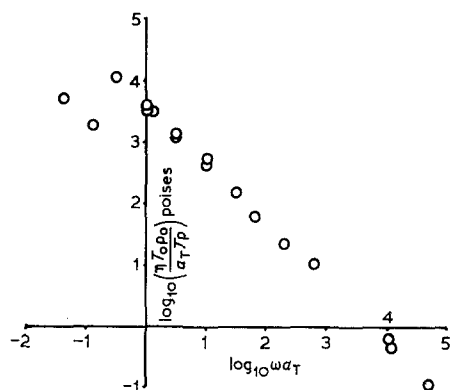*Figure 5* Variation of the viscous component of the modulus, G'', with reduced frequency, ωa_T.

Figure 6 Variation of viscosity, η, with reduced frequency, ωa_T.

3.2. Direct shear

The shapes of the stress-strain curves of all three durations of straining were similar. As may be seen from Fig. 7, which is the curve for $7\frac{1}{2}$ sec straining, the stress built up rapidly for about 2 sec, corresponding to the attainment of a total shear of about 0.5, at which point there was a sudden drop, after which the stress built up again, reaching a higher value than that at which the interruption occurred. When shearing was continued for 15 sec, a maximum was reached after about 8 sec, after which the stress fell slightly. On removing the drive, the stress initially fell rapidly; the course of this relaxation could not be followed as it was faster than the time of response of both the machine and the recorder. However, after about 1 sec, indicated on the diagram by an asterisk, it was evident that the stress had fallen to about half the maximum value, and was relaxing very slowly. When the reverse shear was applied the trace was almost a
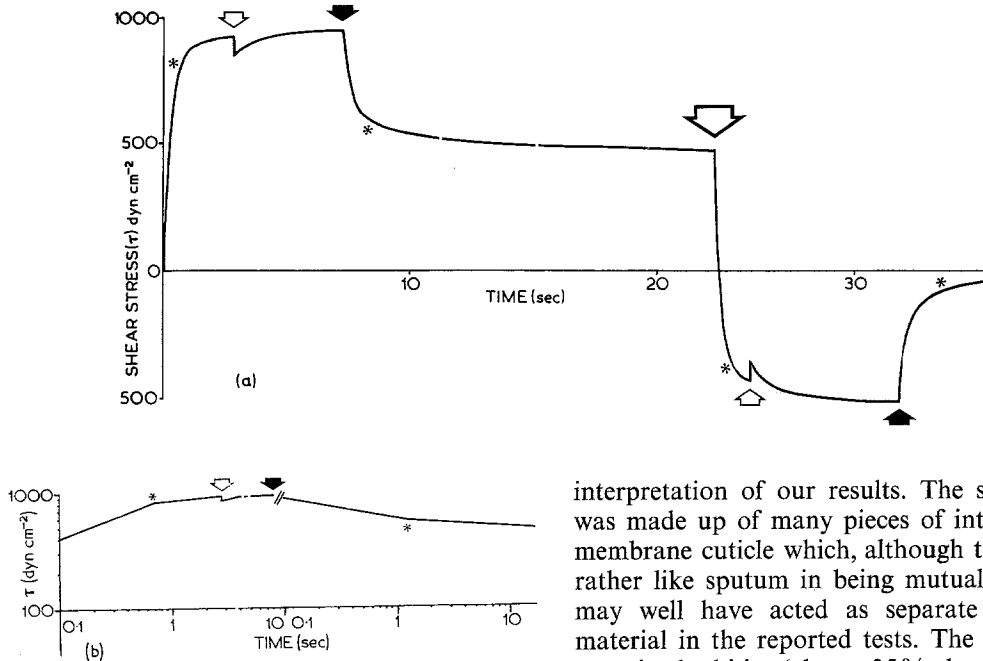
Figure 7(a) Constant shear experiment: transitions in the log-log plot occur at the places marked with an asterisk. The (small) open arrow marks a yield point in the material under shear; the solid arrow marks the cessation of shearing. The large open arrow marks the start of reverse shearing. (b) Log-log plot of Fig. 7a.

mirror image of the forward trace, but with the origin displaced to a positive stress equal to the unrelaxed stress remaining from the forward drive. As a result, after the reverse drive had been removed the stress fell back rapidly towards zero. One forward run was repeated and the stress allowed to relax for a long time. The initial behaviour was as already described, and slow relaxation continued for at least 1 h, by which time the stress had still only fallen to 0.29 of the maximum.

3.3. Effects of extensive shearing
The curve obtained in the sinusoidal mode of operation *after* the drastic unidirectional shearing, was indistinguishable in shape and magnitude from the curve of the type shown in Fig. 1. This demonstrates that the treatment which the sample had undergone did not produce any permanent effect on its rheological properties.

4. Discussion
4.1. Introduction
There are certain reservations to be made in the

interpretation of our results. The sample used was made up of many pieces of intersegmental membrane cuticle which, although they handled rather like sputum in being mutually adhesive, may well have acted as separate packets of material in the reported tests. The sample also contained chitin (about 35% dry wt), which very probably affected the results in some way. However, since ω was kept small, the straining of the specimen in the sinusoidal tests was small compared with the lengths of the individual pieces of cuticle. The above reservations, having been made, are ignored in the interpretation. The more easily comparable of the results do, however, agree with results obtained from linear experiments [3].

4.2. Responses to shearing
The shape of the response curve (Fig. 2) is anomalous, and of a form rarely reported. It has, however, been observed with double cream and mayonnaise [7], which are essentially emulsions. The anomalous shape with these substances is attributed to the heterogeneity of the system, but not to any network of structure. The heterogeneity of the samples used in our experiments is of two orders of size: the first due to pooling cuticle from several locusts, the second, to the presence of chitin. Since we estimate that not more than ten pieces of cuticle were contained in the annular space of the testing machine at any one time during the tests, any effect due to their mutual shearing might be expected to have made the response much less constant. One would expect slippage in each cycle once a certain critical strain was reached. This was not observed even in the very large-amplitude sinusoidal tests, though it may account for the interruption

in the curves obtained in the continuous shear experiments (Fig. 7, open arrows). The chitin is present in the form of long chains, probably assembled into microfibrils; but since these chains are orders of magnitude smaller than emulsion droplets, they probably could not imitate the emulsions for which this anomalous response has been recorded. The possibility remains that the anomalous response is due to the protein fraction of the cuticle alone. We intend to extract the protein fraction of the cuticle and test it on its own.

4.3. Anomalous temperature effect

In Fig. 6, two points at the top end of the curve are obviously off the line formed by the rest of the points. These two points were from data obtained at 40°C, and it is possible that, even at this relatively low temperature, there is some change in the material. This is supported by evidence from linear tests [3] where an increase in the modulus of the material is seen at 70°C, but this increase is observed at much lower temperatures (53°C) if the cuticle is left for 5 h at that temperature. We do not, therefore, consider that these points indicate a plateau in the curve of Fig. 6, but rather that the material has changed at this elevated temperature, which has had the effect of displacing the entire curve as represented by these two points.

4.4. Moduli, viscosity and tan δ

The elastomer is most probably the protein part of the cuticle, which is present as 65% dry wt. This protein is probably present as a high polymer, mol wt $> 10^6$ [3], and is assumed to be the fraction mainly responsible for our results. The protein is uncross-linked in its major part.

However, our results are not at all like those given by Ferry [8] as typical of a high molecular weight, uncross-linked polymer. Using the same co-ordinates for the various relationships, Ferry's data are curvilinear, whilst our data all fit straight lines. More importantly, tan δ is high over an amazingly large range of ωa_T. In general form, our results are much more like those given for a glassy solid. The cuticle is clearly *not* to be considered "glassy" in terms of its modulus, but in terms of a glass's being densely packed; this similarity is a very attractive idea. Apart from the appearance of the relationships shown in Figs. 4 to 6, the following argument gives support to the idea that the "glassy" type of response is due to dense packing

of the elastomer.

Tan δ is remarkably high and constant over a remarkably large range of ωa_T. If tan δ is typically high in transition zones [8], then it follows that Fig. 3 may be interpreted either as evidence of a continuous structural transition or of a series of closely spaced structural transitions. The molecular origin of these transitions will now be discussed.

There is another elastic protein in insects named resilin [9] whose amino-acid composition bears a remarkable relationship to that of the intersegmental membrane cuticle [10]. It seems highly likely that at least part of the elasticity of both materials is derived in some way from specific conformations imposed upon the cuticle protein by a specific sequence of amino-acids. This would demand not only a densely packed protein (far more densely packed, that is, than random coils would be), but a greater contribution of the energy term to the elasticity. Unfortunately, since the relaxed modulus of the material is zero owing to the protein's being uncross-linked, we have not been able to do the easiest of the thermodynamic experiments, so such a study must wait. Then a possible explanation of the continuous state of transition would be to consider each possible interaction between pairs of different amino-acids as capable of providing a single type of transition. There are fifteen amino-acids in resilin and in the intersegmental membrane, so that if each amino-acid is free to interact with another amino-acid of similar type (e.g. non-polar *not* interacting with polar), there will be something like 100 *separate* transitions available.

The protein is then envisaged to be something like extensible polyethylene in folded conformation. When the chain is extended, the folds are pulled apart, resulting in the breaking of the bonding between the amino-acids of the adjacent protein chains. The amino-acids may or may not be solvated. The elastic recovery is aided by thermal agitation to bring the chains together, when it is conceivable that a zip-like action increases the speed of return to the minimum energy state. The model as proposed also accounts for the high initial hysteresis observed, and the rapid reduction in hysteresis loss on continued cycling [3].

4.5. Conclusions

The outstanding properties of the locust intersegmental membrane cuticle are its in-

credible elastic extensibility and its very low modulus. During the course of the experiments reported here, the material was extended to a strain of 9.6 without any sign of damage. In linear tests, strains of 15 have been recorded, and have been fully recoverable. It is not impossible that such properties could be shown by a rubbery polymer but, in that event, the side chains of the amino-acids would have to be adequately masked to prevent interactions between the very long random coils which would be necessary. The insect may well introduce some subtle modifying agent to allow this. It seems to us more likely, however, on what may be considered philosophical grounds, that it would be a great waste of energy for the insect to define, apparently very closely [10], the amino-acids and presumably, therefore, the shape of the elastomer molecule, and not to make use of this information.

It becomes apparent that the molecular theory of elasticity proposed here for this unusual material is a new one (to the best of our knowledge) and may well be applicable to the elastic behaviour of other apparently amorphous proteins. Thus this model is not suitable as an explanation of how collagen or keratin work, but could be applied to low modulus proteinaceous substances such as sputum. It is encouraging, therefore, that Davis [11] shows, in his Fig. 5, results obtained with sputum which are very like our own, obtained with insect cuticle.

Acknowledgements

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