

## RUBBER-LIKE PROPERTIES OF THE INNER HINGE-LIGAMENT OF PECTINIDAE

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### INTRODUCTION

The inner hinge-ligament of Pectinidae (Lamellibranchiata) possesses long-range elasticity, and serves as the elastic antagonist of a muscle which is used in swimming. This paper is concerned with the molecular basis of this elasticity, and with the efficiency with which the ligament performs its function. It follows a paper by Trueman (1953).

The hinge ligament consists of two parts (Trueman, 1953, figs. 1, 2). The outer part holds the two valves closely together along the hinge line. It is flexible, and acts as a hinge. The inner part is a block of elastic material which acts as a compression spring, making the shell gape when the adductor muscle relaxes. It is calcified at its lateral ends where it is attached to the valves, but its main part is an uncalcified protein. It breaks with a conchoidal fracture, and shows no birefringence in sections (of *Pecten*) hand-cut in three planes mutually at right-angles. It is therefore amorphous, at least when unstrained. The excised ligament bounces like rubber when dropped on a hard surface.

### *Thermodynamics of protein elasticity*

Various structural proteins, including the inner hinge-ligament, show long-range elasticity. In keratin this involves transformation between two crystalline states (Ciferri, 1963). In most other cases, it is probably similar in nature to the long-range elasticity of rubber, in which changes of entropy are more important than changes of internal energy. That is to say, the elastic restoring force is probably mainly due to the tendency of long, thermally agitated molecules to take up a random configuration.

Consider a specimen which reaches equilibrium at length  $l$  when stretched by a force  $f$ . Let its volume, temperature and entropy be  $V$ ,  $T$  and  $S$ , respectively. It can be shown that

$$(\partial S/\partial l)_{V, T} = -(\partial f/\partial T)_{V, l} \quad (1)$$

(see, for instance, Treloar, 1949). It is therefore possible to calculate the component,  $-T(\partial S/\partial l)_{V, T}$ , of the elastic restoring force at given length, which is due to entropy change, if the force is determined at more than one temperature without allowing the volume of the sample to change.

It is highly inconvenient to apply the pressures necessary to keep the volume of the sample constant, though this has been done in experiments on rubber (Allen, Bianchi & Price, 1963). Fortunately the volume of unswollen amorphous polymers changes little with temperature, and values obtained at constant pressure can be used in any but the most precise determinations of the entropy component of the restoring force.

Weis-Fogh (1961*b*) showed, by an analysis of this type, that the elasticity of an insect protein, resilin, is mainly associated with entropy changes. Other proteins whose long-range elasticity has been investigated (see Weis-Fogh, 1961*b*) change their degree of swelling as the temperature changes. This greatly complicates the thermodynamic analysis (Oplatka, Michaeli & Katchalsky, 1960; Hoeve & Flory, 1962). Hoeve & Flory (1958) avoided this difficulty in the case of elastin by performing their experiments in a mixture of water and glycol in which the degree of swelling did not change with temperature. They concluded from their results that the elasticity of elastin is almost entirely due to entropy changes. Their procedure has been criticized (Oplatka *et al.* 1960) and defended (Hoeve & Flory, 1962).

Resilin and elastin seem to be the only proteins in which rubbery elasticity has been demonstrated. Trueman (1953), in experiments with Pectinidae, found that the force required to hold the shell at a given angle of gape increased as the temperature was raised. This suggests that  $(\partial f/\partial T)_l$  for the inner hinge-ligament is positive, and that the elasticity of the inner ligament may be rubber-like. However, Trueman also performed experiments on excised inner hinge-ligaments, and found no effect of temperature on elastic restoring force. He therefore concluded that the effect he observed with the ligament in place in the shell was due to contraction of the outer layer of the ligament at higher temperatures. Evidence presented in this paper indicates, however, that the elasticity of the inner hinge-ligament is in fact mainly associated with entropy changes.

### *Resilience*

A confusing variety of measures of efficiency of elastic behaviour are, unfortunately, in use. The one which seems best suited to my purpose is resilience. This is the ratio of the work done by a specimen in elastic recoil to the work done on it when it was previously strained, expressed as a percentage. If the specimen, with or without an added load, is set in free oscillation, and the amplitudes at two successive peaks (half a cycle apart) are  $x_n$ ,  $x_{n+1}$ , the resilience is  $100(x_{n+1}/x_n)^2$ . Resilience is most commonly used to give the results of rebound experiments. Values obtained from rebound experiments are only comparable to those obtained from oscillation experiments when the time of contact in the former experiments is about half the period in the latter ones. Some authors give other meanings to 'resilience' (Love, 1893; Gehman, Woodford & Stambaugh, 1941).

The loss tangent (Leaderman, 1957) is a quantity extensively used to express the results of oscillation experiments. It is the tangent of the phase angle between stress and strain in forced oscillation. It is also the ratio of the loss modulus to the storage modulus. It is  $2/\pi$  times the logarithmic decrement per half cycle for free oscillations,  $\log_e(x_n/x_{n+1})$  (see Ferry, 1961, where, however, the logarithmic decrement *per cycle* is used).

The resilience,  $R$ , loss tangent,  $\tan \delta$ , and logarithmic decrement per half cycle,  $\Delta$ , are thus related by the equation

$$\log_e(100/R) = \pi \tan \delta = 2\Delta. \quad (2)$$

Trueman (1953) published hysteresis loops for the opening and closing of the valves of a variety of lamellibranchs. The resilience of the hinge ligaments can be

calculated from the areas under the loading and unloading curves. It is 90% or more for Pectinidae, and 80% or less for some other lamellibranchs. Trueman attributed the difference to the fact that the inner hinge-ligaments of Pectinidae are calcified only at their ends, while those of other lamellibranchs are calcified throughout. He correlated the difference with the swimming habit of the Pectinidae. They swim by rapid opening and closing of the valves (Yonge, 1936; Baird, 1958). High resilience of the ligament is plainly advantageous because it reduces the energy required for swimming. The other lamellibranchs studied by Trueman do not swim.

The resilience of a polymer varies with frequency. The cycles of loading and unloading in Trueman's experiments apparently took several minutes each. Swimming Pectinidae open and close their valves several times per second. The resilience has now been determined at about the frequency of the swimming movements.

#### MATERIALS AND METHODS

*Pecten maximus* (L.) and *Chlamys opercularis* (L.) were used in the experiments. These were the species used by Trueman (1953). Some of the specimens were fresh, some had been preserved in 5% formaldehyde for up to 8 days and some had been eviscerated, stored dry for a few weeks and soaked in water for several days before use. No differences were observed between the properties of ligaments treated in these different ways, but the histories of the ligaments used are given in the descriptions of method which follow.

#### *Thermodynamic experiments with the ligament in the shell*

*Pecten* was used for these experiments. Some of the specimens (numbers 1 and 2 of Table 1) were fresh, and the others had been preserved in formaldehyde.

All parts of the body, other than the ligament, were removed from the shell. A hole was bored through the convex valve near its ventral edge in the plane of symmetry of the shell. A large notch was ground in the ventral edge of the flat valve. The flat valve was clamped to a heavy brass frame, and frame and shell were immersed in a thermostatically controlled bath filled with tap water (Fig. 1). The hole in the convex valve was used for the suspension of a pan for weights, and for the attachment of a thread leading to the recording lever. This lever bore a swinging glass stylus which wrote on a smoked kymograph drum, recording changes in the gape of the shell. The notch in the flat valve made room for the weight pan. The lever was adjusted so that it balanced when the empty pan, immersed in water, was suspended from it.

A compressive force due to the weight of the convex valve always acted on the ligament. This was taken to be equivalent to half the weight in water of the valve, acting at the point of suspension of the weight pan. This quantity was added to the sum of the weights in water of the weights in the pan to obtain the loads indicated in Table 1 and Fig. 3. The weights each weighed 13.5 g. in water.

The displacements indicated in Fig. 3 are vertical displacements from an arbitrary zero of the point of suspension of the weight pan, obtained from the kymograph record. The displacement was recorded at each temperature with 0, 1, 2, 3, 4 and 0 weights in the pan, in that order. The weights were changed at 2 min. intervals, and each record was taken just before the next change in weight was due. The first of the

two values with 0 weights in the pan was used for plotting the graphs, but the second was on average only 0.03 cm. higher.

Load-displacement curves were obtained in this way at a high temperature (67–80° C.), at one or more lower temperatures, and then at the high temperature again. The temperature was usually changed rapidly by replacing the water in the bath with water at the required temperature. No readings were taken until the bath had been at the required temperature for at least 20 min., but longer delays at high temperatures were avoided.

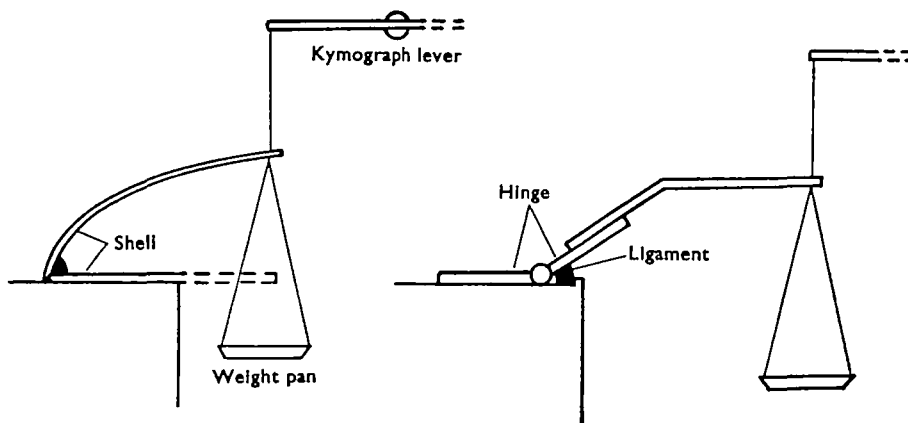


Fig. 1

Fig. 2

Fig. 1. The apparatus for the thermodynamic experiments with ligaments in their shells.

Fig. 2. The apparatus for the experiments with excised ligaments.

It was found that the gape of the shell increased (i.e. negative displacement occurred) when it was kept at high temperatures with no weights in the pan. This effect is thought to have been due to contraction of the outer ligament, as it was not observed in experiments with isolated inner ligament. The rate of increase was only about 0.1 cm./hr. at 70° C., so its effect on the results was negligible, but it was necessary to avoid keeping the specimens at high temperatures for unduly long periods.

The load-displacement curves at different temperatures were used to obtain  $(\partial f/\partial T)$ , for various displacements, and from these the entropy components of the elastic restoring force were calculated, as has been explained.

#### *Thermodynamic experiments with excised ligaments*

*Pecten* ligaments which had been stored dry were used in these experiments. The apparatus is shown in Fig. 2. One leaf of a steel backflap hinge is screwed to a heavy base. An iron bar 12 cm. long is bolted to its other leaf. The excised inner hinge-ligament is compressed between the free leaf of the hinge and a steel plate which tops the base. A small flange on this plate prevents the ligament from sliding further from the axis of the hinge. Weights are suspended, as required, from the free end of the iron bar, and movements of this end are recorded on a kymograph drum.

The procedure in all but one of the experiments was the same as with ligaments in their shells, except that two increments of weight were used instead of four, and heavier weights were used.

A different procedure was used, in a single experiment, to satisfy an objection to the other experiments which is raised in the Discussion section of this paper. A load was applied and the specimen was kept in the apparatus at 67° C. for 23 hr. The position of the bar was then recorded on the kymograph drum. The temperature was rapidly lowered to 27° C. by changing the water, and maintained at 27° for 1 hr. Another record was then made. The load was changed and the procedure repeated.

#### *Creep experiments*

The relevance of these experiments is explained in the Discussion section of this paper. Two fresh *Pecten* and one formalin-preserved one were used in experiments at 20° C., and two preserved ones at 70° C. The ligaments were mounted in the same way as in the thermodynamic experiments on ligaments in their shells, and creep after the addition of 54 g. to the pan was recorded on the kymograph drum.

#### *Determination of resilience*

The resilience of the hinge ligament was determined by following the decay of free oscillations of one valve, while the other valve was firmly held. Fresh specimens of *Chlamys* were used. All parts of the body, other than the ligament, were removed from the shell. Anterior and posterior segments totalling about 60% of the area of the valve were ground off the valve which was to be allowed to oscillate, to reduce the effect of air resistance. The other valve was clamped firmly to the bench. A fragment of mirror was attached to the free valve, near the hinge, by means of Plasticine. A lump of Plasticine was applied to the ventral margin of the free valve to reduce its resonant frequency to about 4 cyc./sec. This is reasonably near to the frequency of movement of the valves in swimming which I estimate, from observations of specimens in an aquarium, to be about 3 cyc./sec. A beam of light was projected on to the mirror, which reflected it on to a screen. The free valve was made to oscillate, and the consequent vertical movements of the light spot on the screen were recorded by means of a camera, which was turning slowly about a vertical axis. A very similar technique was used by Jensen & Weis-Fogh (1962) in experiments on the hind tibia of *Schistocerca*. The logarithmic decrement was obtained from the gradient of a graph of the logarithm of amplitude against the serial number of the peak (Fig. 5) and was used to calculate the resilience (equation 2).

#### RESULTS

The results of the experiments with ligaments in their shells are shown in Fig. 3 and Table 1. In every case, the load-displacement curves for different temperatures would intersect, if produced, at about zero load. This indicates that the degree of swelling is not appreciably affected by temperature changes within the range used in the experiments. We have seen that this is a prerequisite for the thermodynamic analysis. The initial and final load-displacement curves for each specimen at the higher temperature agreed closely, though not always as exactly as in the example of Fig. 3. There is good agreement between the load and the estimated entropy component of the elastic restoring force except at 15 g. load, where the entropy component is too high. The agreement suggests that we are dealing with a case of rubber-like elasticity.

The results of the experiments with excised inner hinge-ligaments, in which the load was varied while the temperature was kept constant, are summarized in Table 2. They are very similar to the results obtained with ligaments in their shells. The estimated entropy component agrees reasonably well with the load except at 30 g. load, where it is too high. The stress on the ligament at 30 g. load in these experiments was about the same as the stress at 15 g. load in the experiments with ligaments in their shells, owing to a difference in mechanical advantage. The agreement between Tables 1 and 2 shows that the properties of the outer hinge-ligament did not affect the results significantly in the experiments with ligaments in their shells.

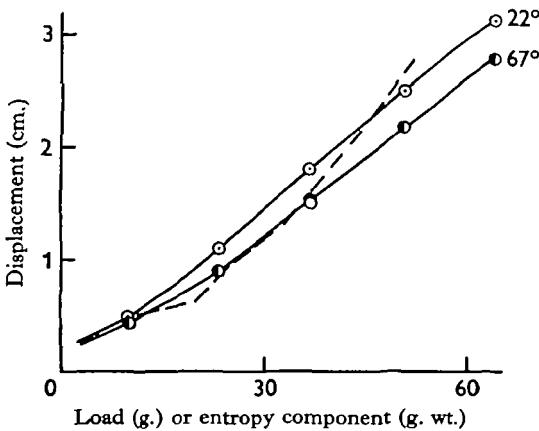


Fig. 3

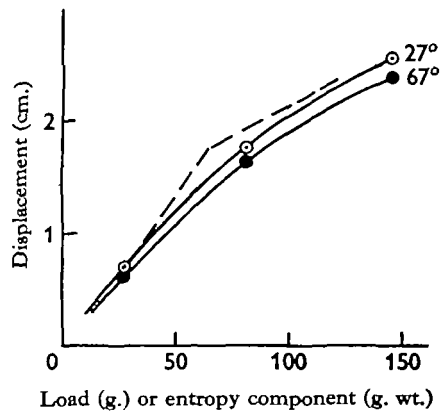


Fig. 4

Fig. 3. The results of a thermodynamic experiment with a ligament in its shell. The continuous lines are graphs of displacement against load, from sets of readings taken at 67° (○), 22° (○) and finally again at 67° C. (●). The broken line is a graph of displacement against the estimated entropy component of the elastic restoring force at 22° C.

Fig. 4. The results of the experiment with an excised inner hinge-ligament in which the load was kept constant while the temperature was changed. The continuous lines are graphs of displacement against load at 27° (○) and 67° C. (●). The broken line is a graph of displacement against the estimated entropy component of the elastic restoring force at 27° C.

Table 1. *The results of the thermodynamic experiments with ligaments in their shells*

Load (g.)	Estimated entropy component (g. wt.)					Mean
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	
15	24	33	28	21	18	25
30	32	37	38	34	30	34
50	47	52	58	49	46	50

Table 2. *The results of the thermodynamic experiments with excised ligaments, in which the load was varied at constant temperature*

Load (g.)	Estimated entropy component (g. wt.)			Mean
	Expt. 1	Expt. 2	Expt. 3	
30	46	52	60	53
80	76	100	94	90
125	131	107	108	115

The results of the single experiment with an isolated ligament, in which the temperature was changed while the load was kept constant, are shown in Fig. 4. There is good agreement between the estimated entropy component and the load at the same length.

The load-displacement curves for ligaments in their shells (e.g. Fig. 3) are slightly concave, while those for excised ligaments (e.g. Fig. 4) are convex. The difference is probably entirely due to the geometry of the apparatus. The recorded displacements are vertical movements of the points of attachment of the weights, while the changes in the length of the ligaments were proportional to the angular movements of these points. The free leaf of the hinge in the experiments with excised ligaments moved through smaller angles than did the free valve in the experiments with ligaments in their shells.

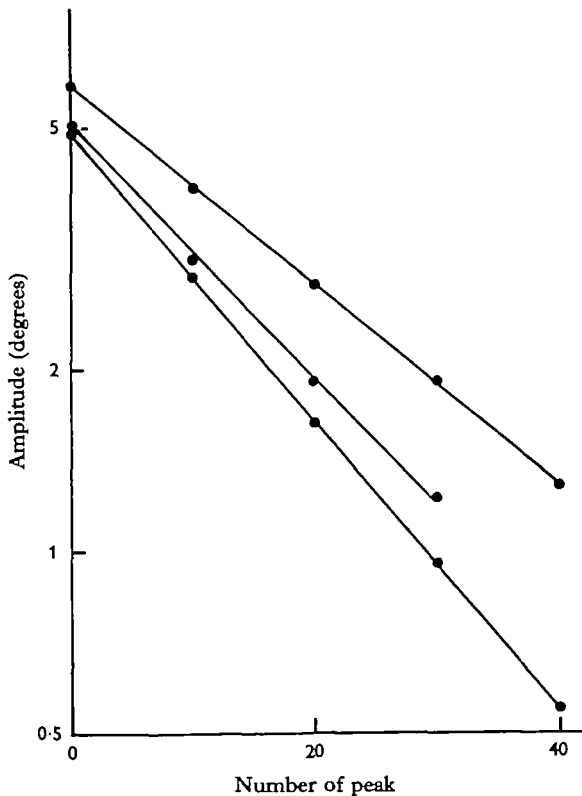


Fig. 5. Graphs of amplitude (on a logarithmic scale) against the serial number of the peak (half-cycle) in the decay of free oscillations in the experiments with *Chlamys*.

No attempt was made to correct the results for thermal expansion; they are not accurate enough to justify this.

Creep was followed for 18 hr. after loading in experiments at 20° C., and for 3 hr. in experiments at 70° C. Most of the displacement occurred very rapidly (within 3 sec.), but constant length was not attained. Graphs of displacement against the logarithm of time were very nearly straight lines, from 3 sec. after loading to the ends of the experiments. The strain increased by about 5% for every factor of 10 of time at 20° C., and by about 8% for every factor of 10 of time at 70° C.

The shape of the inner hinge-ligament makes the determination of Young's modulus difficult, but I estimate from my results that it is about  $4 \times 10^7$  dynes/cm.<sup>2</sup>. This is, of course, a pseudo-equilibrium modulus, since very slow creep continued to the ends of the experiments. Trueman (1953) estimated Young's modulus to be  $3 \times 10^7$  dynes/cm.<sup>2</sup>.

The results of the free oscillation experiments are shown in Fig. 5. The resiliences calculated from them are 90, 91 and 93%. Higher values would probably have been obtained if air resistance had been eliminated since a preliminary experiment with one of the specimens, before the area of the oscillating valve had been reduced by grinding, gave a value of only 86%. The mean value for resilience, 91%, corresponds to a loss tangent of 0.03 (equation 2). The frequency was about 4 cyc./sec. This indicates a creep rate of 11% of the strain per factor of 10 of time at times of the order of 0.1 sec. (Ferry, 1961, chapter 4, equation 49). This is twice the rate found at longer times in the creep experiments. The difference is likely to be partly due to errors due to air resistance, but there may well be a change in the retardation spectrum between 0.1 and 3 sec., or a difference between *Chlamys* and *Pecten*.

#### DISCUSSION

##### *The thermodynamic analysis*

Equation 1, which is used for determining the entropy component of the elastic restoring force, refers to measurements of the force at equilibrium. It is plain from the creep experiments that the ligaments were not at equilibrium when readings were taken. The inner hinge-ligament, like rubber and synthetic polymers in the pseudo-equilibrium zone (Ferry, 1961), continues to creep slowly at very long times. This would not affect the analysis if equal length when readings were taken implied equal length at equilibrium. It can, however, be argued that readings at higher temperatures were nearer to the supposed equilibrium than readings at lower temperatures, because increases of temperature shift the spectra of retardation times of polymers to shorter times (Ferry, 1961). The estimated entropy components might thus be too low.

The amount by which the retardation spectrum shifts with temperature can often be determined by matching the shapes of creep curves (plotted against the logarithm of time) for different temperatures, or by means of a calculation which requires knowledge of the glass transition temperature (Ferry, 1961). Neither of these methods is available to us. The creep curves are featureless, and the ligament is still soft at the freezing-point of water, so its glass transition temperature cannot be determined. If the virtual glass transition temperature were close below 0° C., the shift of the retardation spectrum between 20 and 70° C. could be as much as a factor of  $10^5$  of time. This would result, at a creep rate of 5% per factor of 10 of time, in an error of 25% in the displacements in the majority of experiments, in which the temperature was kept constant while the load was varied. Correction for this would about double the estimated entropy components, spoiling the agreement between them and the loads.

In the single experiment in which the load was kept constant while the temperature was changed, errors due to different degrees of equilibration at different temperatures



would not affect the large displacements due to change of load, but only the much smaller ones due to change of temperature. The maximum possible effect on the estimated entropy components would be much less than in the other experiments. Since reasonable agreement between estimated entropy component and load was found in this experiment, as in the others, there can have been no serious errors due to shifting of the retardation spectrum with changes of temperature.

The results of the experiments thus indicate that the elasticity of the inner hinge-ligament of *Pecten* is true rubber-like elasticity, in that the elastic restoring force is mainly associated with entropy changes. They differ from the results of Trueman (1953), who found no change of elastic restoring force with temperature in his experiments with isolated ligaments. As Trueman did not publish his negative results in detail, I am unable to explain why his findings differed from mine.

#### *Comparison with other proteins*

Weis-Fogh (1960) has compared elastin and the insect protein resilin. It seems worthwhile to extend the comparison to include the inner hinge-ligament of Pectinidae.

Elastin occurs in many tissues in vertebrates. One of the sites in which its properties are most fully exploited is the wall of the thoracic part of the aorta of mammals. This part of the aorta is highly elastic (Bergel, 1961a) on account of its high elastin content (Harkness, Harkness & McDonald, 1957). It swells during systole (McDonald, 1953) and recoils during diastole, expelling blood which has accumulated in it into more distal arteries. It presumably thus reduces the effective peripheral resistance. The frequency of the heart beat varies, in different mammals, from about 0.5 to 10/sec. (Young, 1957). Resilin in various structures in the thorax of insects absorbs the kinetic energy of the wings during the upstroke and releases it in the subsequent downstroke (Weis-Fogh, 1961a). It saves energy by transforming kinetic energy in one direction into kinetic energy in the other. The frequency of the wing beat is about 20/sec. in *Schistocerca*, whose resilin was used in Jensen & Weis-Fogh's (1962) study of mechanical properties. The inner hinge-ligament of Pectinidae is the antagonist of the shell adductor muscle. It is compressed when the adductor contracts, and recoils to open the shell when the adductor relaxes. The frequency of the cycles in swimming *Chlamys* is about 3/sec.

Elastin, resilin and inner hinge-ligament are thus used for very similar purposes. All are subject to cycles of strain and release. Work is done on them, storing elastic energy which is subsequently used, in the elastic recoil, to do useful work. The frequency of the cycles lies, in the cases which have been most studied, between 1 and 20/sec.

The similar functions of the three materials require similar mechanical properties. The materials must be elastic. They must not be liable to purely viscous flow or to permanent set due to crystallization brought about by strain (see, for instance, Treloar, 1940); if they were, their dimensions would change progressively in use. The resilience of each material must be as high as possible in the working range of frequencies.

These requirements are satisfied in each case by a cross-linked amorphous polymer, which is in its pseudo-equilibrium zone (see Ferry, 1961) at the temperature and frequency concerned. All are proteins plasticized with water (resilin contains about

55% water, Weis-Fogh, 1960; *Pecten* inner hinge-ligament contains about 50%). Elastin (Cox & Little, 1961) and resilin (Weis-Fogh, 1961c), at least, can suffer substantial strains without crystallization.

Cross-linked polymers show no viscous flow. If they are not highly crystalline, and do not contain too high a proportion of plasticizer, they usually have pseudo-equilibrium moduli of the order of  $10^{-7}$  dynes/cm.<sup>2</sup>. The precise value depends on the density and arrangement of the cross-links (Flory, 1956, equation 44). Young's modulus is about  $6 \times 10^6$  dynes/cm.<sup>2</sup> for elastin (Bergel, 1961a),  $2 \times 10^7$  for resilin (Weis-Fogh, 1961c; shear modulus =  $7 \times 10^6$ ) and  $4 \times 10^7$  for *Pecten* inner hinge-ligament. The elasticity of amorphous polymers above their glass transition temperatures is mainly due to changes of entropy with length: this has been shown to be the case for elastin (Hoeve & Flory, 1958), resilin (Weis-Fogh, 1961b) and *Pecten* inner hinge-ligament (this paper).

The resilience of cross-linked amorphous polymers in the pseudo-equilibrium zone is very high, around 90% (loss tangent about 0.03: see Ferry, 1961). The resilience of resilin is 97% in its working range of frequencies (Jensen & Weis-Fogh, 1962) and that of *Chlamys* inner hinge-ligament at least 91% (this paper). The resilience of elastin does not seem to have been determined but must be high; Bergel's (1961b) values for phase lag show that the resilience of the wall of the thoracic aorta at 2-4 cyc./sec. is about 80%.

Though the materials we have been comparing are so similar in features which concern their mechanical properties, they differ in other features. The cross-linkages of elastin involve pyridine rings (Thomas, Elsdon & Partridge, 1963; Bedford & Katritzky, 1963), those of resilin are dityrosine and trityrosine (Andersen, 1964) and those of inner hinge-ligament are presumably formed by quinone tanning in Pectinidae as in other lamellibranchs (see Beedham, 1958). Bailey & Weis-Fogh (1961) have pointed out the striking differences in amino-acid composition between elastin and resilin. The inner hinge-ligaments of a variety of lamellibranchs differ from both elastin and resilin in being rich in methionine (Beedham, 1958), but no analyses have been done on Pectinidae, and the ligaments which have been analysed are very different in mechanical properties from those of Pectinidae (Trueman, 1953).

The elastic materials which have been discussed are very different from the mesogloea of sea anemones (Alexander, 1962) and the tunica externa of the swimbladder of Cyprinidae (Alexander, 1961). These show long-range elasticity, but most of their compliance is associated with high retardation times. This makes the mesogloea less compliant to brief stresses than it would otherwise be, and enables the tunica externa to act as a frequency filter.

#### SUMMARY

1. The inner hinge-ligament of Pectinidae (Lamellibranchiata) is an elastic block of amorphous cross-linked protein, plasticized with water. It acts as a compression spring, and is the antagonist of the shell adductor muscle.

2. Its elasticity is shown to be rubber-like, in that the elastic restoring force is mainly due to entropy changes. Rubber-like elasticity has been demonstrated by other authors in two other proteins (elastin and resilin).

3. Pectinidae swim by rapid opening and closing of the shell. The resilience of the inner hinge-ligament of *Chlamys* at the frequency of the swimming movements is at least 91%.

4. The inner hinge-ligament of Pectinidae is compared with other proteins which show long-range elasticity.

I am grateful to Dr N. W. Runham for introducing me to the hinge-ligament and for information about it.

## REFERENCES

- ALEXANDER, R. McN. (1961). Visco-elastic properties of the tunica externa of the swimbladder in Cyprinidae. *J. Exp. Biol.* **38**, 747-57.
- ALEXANDER, R. McN. (1962). Visco-elastic properties of the body-wall of sea anemones. *J. Exp. Biol.* **39**, 373-86.
- ALLEN, G., BIANCHI, U. & PRICE, C. (1963). Thermodynamics of elasticity of natural rubber. *Trans. Faraday Soc.* **59**, 2493-502.
- ANDERSEN, S. O. (1964). The cross-links in resilin identified as dityrosine and trityrosine. *Biochim. Biophys. Acta*, **93**, 213-15.
- BAILEY, K. & WEIS-FOGH, T. (1961). Amino acid composition of a new rubber-like protein, resilin. *Biochim. Biophys. Acta*, **48**, 452-9.
- BAIRD, R. H. (1958). On the swimming behaviour of scallops (*Pecten maximus* L.). *Proc. Malac. Soc.* **33**, 67-71.
- BEDFORD, G. R. & KATRITZKY, A. R. (1963). Proton magnetic resonance spectra of degradation products from elastin. *Nature, Lond.*, **200**, 652.
- BEEHAM, G. E. (1958). Observations on the non-calcareous component of the shell of the Lamellibranchia. *Quart. J. micr. Sci.* **99**, 341-57.
- BERGEL, D. H. (1961*a*). The static elastic properties of the arterial wall. *J. Physiol.* **156**, 445-57.
- BERGEL, D. H. (1961*b*). The dynamic elastic properties of the arterial wall. *J. Physiol.* **156**, 458-69.
- CIFERRI, A. (1963). The  $\alpha\rightleftharpoons\beta$  transformation in keratin. *Trans. Faraday Soc.* **59**, 562-9.
- COX, R. C. & LITTLE, K. (1965). An electron microscope study of elastic tissue. *Proc. Roy. Soc. B*, **155**, 232-42.
- FERRY, J. D. (1961). *Viscoelastic Properties of Polymers*. New York: Wiley.
- FLORY, P. J. (1956). Theory of elastic mechanisms in fibrous proteins. *J. Amer. Chem. Soc.* **78**, 5222-35.
- GEHMAN, S. D., WOODFORD, D. E. & STAMBAUGH, R. B. (1941). Dynamic properties of rubber. Dependence on pigment loading. *Ind. Engng Chem.* **33**, 1032-8.
- HARKNESS, M. L. R., HARKNESS, R. D. & McDONALD, D. A. (1957). The collagen and elastin content of the arterial wall in the dog. *Proc. Roy. Soc. B*, **146**, 541-51.
- HOEVE, C. A. J. & FLORY, P. J. (1958). The elastic properties of elastin. *J. Amer. Chem. Soc.* **80**, 6523-6.
- HOEVE, C. A. J. & FLORY, P. J. (1962). Elasticity of crosslinked amorphous polymers in swelling equilibrium with diluents. *J. Polymer. Sci.* **60**, 155-64.
- JENSEN, M. & WEIS-FOGH, T. (1962). Biology and physics of locust flight. V. Strength and elasticity of locust cuticle. *Phil. Trans. B*, **245**, 137-69.
- LEADERMAN, H. (1957). Proposed nomenclature for linear visco-elastic behaviour. *Trans. Soc. Rheol.* **1**, 213-22.
- LOVE, A. E. H. (1893). *A Treatise on the Mathematical Theory of Elasticity*, vol. 2. Cambridge University Press.
- MCDONALD, D. A. (1953). Lateral pulsatile expansion of arteries. *J. Physiol.* **119**, 28 P.
- OPLATKA, A., MICHAELI, I. & KATCHALSKY, A. (1960). Thermoelasticity of open systems. *J. Polymer Sci.* **46**, 365-74.
- THOMAS, J., ELSDEN, D. F. & PARTRIDGE, S. M. (1963). Partial structure of two major degradation products from the cross-linkages of elastin. *Nature, Lond.*, **200**, 651-2.
- TRELOAR, L. R. G. (1940). Elastic recovery and plastic flow in raw rubber. *Trans. Faraday Soc.* **36**, 538-49.
- TRELOAR, L. R. G. (1949). *The Physics of Rubber Elasticity*. Oxford. Clarendon Press.
- TRUEMAN, E. R. (1953). Observations on certain mechanical properties of the ligament of *Pecten*. *J. Exp. Biol.* **30**, 453-67.
- WEIS-FOGH, T. (1960). A rubber-like protein in insect cuticle. *J. Exp. Biol.* **37**, 889-907.

- WEIS-FOGH, T. (1961 *a*). Power in flapping flight. In *The Cell and the Organism*, pp. 283-300. Ed. J. A. Ramsay and V. B. Wigglesworth. Cambridge University Press.
- WEIS-FOGH, T. (1961 *b*). Thermodynamic properties of resilin, a rubber-like protein. *J. Mol. Biol.* **3**, 520-31.
- WEIS-FOGH, T. (1961 *c*). Molecular interpretation of the elasticity of resilin, a rubber-like protein. *J. Mol. Biol.* **3**, 648-67.
- YONGE, C. M. (1936). The evolution of the swimming habit in the Lamellibranchia. *Mém. Mus. Hist. nat. Belg.* (2) **3**, 77-99.
- YOUNG, J. Z. (1957). *The Life of Mammals*. Oxford: Clarendon Press.