

ON THE MECHANICAL PROPERTIES OF THE HINGE LIGAMENT OF *ANODONTA CYGNEA* L. (PELECYPODA) IN SITU

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The importance of hinge-ligament of mussels in the functioning of adductors seems to be evident on the basis of a simple observation. Namely, after cutting the adductors, the shells open up even by an angle of 50 degrees and a significant force is necessary to close them.

For this reason in investigating the adductor activity but more especially its relaxation when this factor is neglected beside considering the properties of the muscle and the nervous control, serious errors can be committed. That is to say the forces developed in the ligament are comparable with those of the adductor.

A detailed mechanical and morphological analysis was carried out by TRUEMAN (1949; 1950; 1951; 1953) on the ligament of *Pecten maximus* and other marine clams. The author described that the *Pecten*-ligament consists of an rigid outer and an elastic inner part. The energy responsible for the opening force is stored when the latter is compressed during the closure of the shells. Some observation on the ligament of *Anodonta cygnea* were also carried out by TRUEMAN (1953). His results are mainly of steady state character even for *Pecten* and must be completed in the case of *Anodonta*. For the latter a mechanical model cannot be presented, because the value of Young modulus and the time-course of the loading-curves are not known.

The results presented here concern the response of ligament at different loading and time in order to compare and mainly to describe the ligament-loading of the adductors in a more dynamic sense.

Method

The experiments were carried out on specimens of constant size of *Anodonta cygnea* L. The preparation of the ligament was carried out by removing all the organs from the shells. The lower shell was fixed horizontally by a weight of 1.5 kg. A hole was drilled in the upper shell and it was attached to a frontal-lever by thread. In this way the ligament was loaded by the recorder-weight of less than 10 g in the direction of opening. The proper weight of the

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shell just counterbalanced it because the weight of the freely moving shell was 21.4 ± 7.7 g (25 cases) and its centre of gravity was about two times nearer to the axis of rotation than the hole drilled for recording and loading.

During the experiments both surfaces of the ligament were protected against running dry by a wet cotton-wool.

The projective distorsion of the recorder lever in the range of its application was less than 3 per cent.

The experiments were carried out by serial loading (5–15 different weights). The weights were hung to a rigid wire at the point drilled in the upper shell (*Figs 1 and 2, Q* — point). The responses obtained after loading were recorded by a kymograph. In this way a time-course of the amplitude was recorded up to 600 sec after the onset.

The results originate from experiments on 25 preparations. At the same time the geometrical data summarized in *Fig. 2* were also measured and/or calculated.

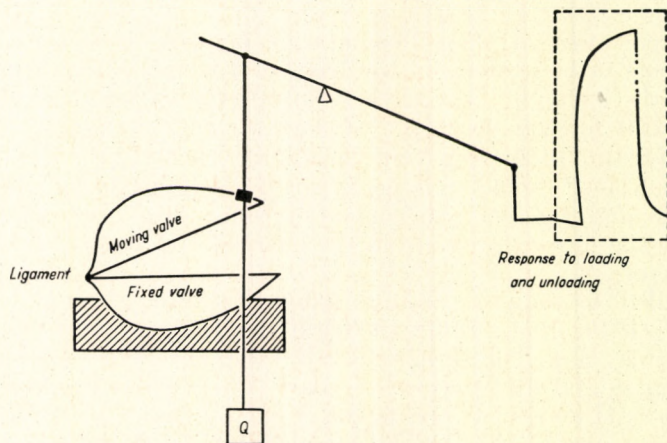


Fig. 1. Experimental arrangement to study the ligament-response to loading

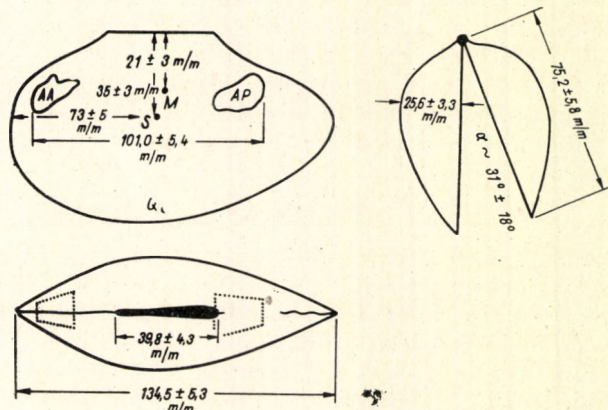


Fig. 2. Geometrical data for the 25 ligament-preparations. Designations: *Q* = point of loading; *S* = projection of the centre of gravity of the shell (its coordinates are also given); *M* = muscle point (see in the text); *AA* = adductor anterior; *AP* = adductor posterior

Results

1. Time course of the ligament response at constant loading

If the loading force attacks at a middle point of the shell edge, a weight of 10 g can already evoke an appreciable displacement and an application of 80—650 g, after a sufficient period causes a total closure. In *Fig. 3* the response is demonstrated when the ligament was loaded by a weight evoking an average-displacement. It is noticeable that a quick displacement — taking place in

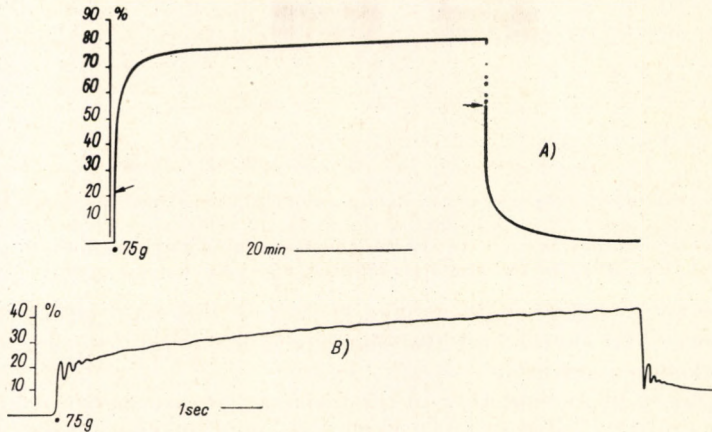


Fig. 3. Time course of the shell displacement when the loading weight was 75 g at the Q-point. A and B = the response of the same animal at different velocity of kymograph

less than 1 sec — is followed by a slow change, approaching its saturation only after some minutes. The loading-off effect also starts with a sudden response and is followed by a slow relaxation. The separation between the two phases is sharp. At this point a damped oscillation is often observable. This oscillation of about 5 cps — in spite of its fairly constant frequency — cannot be taken as being exclusively characteristic of the ligament. Nevertheless, it can be stated — on the basis of some experiments carried out by rigid lever — that the ligament is able to oscillate by a frequency near to 30 cps after having liberated by removing the loading weight. This oscillation dies down exponentially (*Fig. 4A*).

The displaced system, when it is left to move freely by ceasing its loading, generally relaxes in 10—20 min near to the initial level. Nevertheless, it was often necessary before new weightings to overopen the shell by an insignificant force in order to return it to the original line. This did not influence the responses evoked by repeated loads. Responses, however, are disturbed when the weights are applied too often (in intervals of 1—2 min). This effect manifests itself in changes of the separation point of the quick and slow response and of the relaxations reached in a fixed period (*Fig. 4A*). It proved to be certain, that such trace-like, slowly disappearing phenomena are reversible only by external intervention, in spite of the fact that ligament was kept wet. The response of ligament often does not reach its real steady state even after 1 hour.

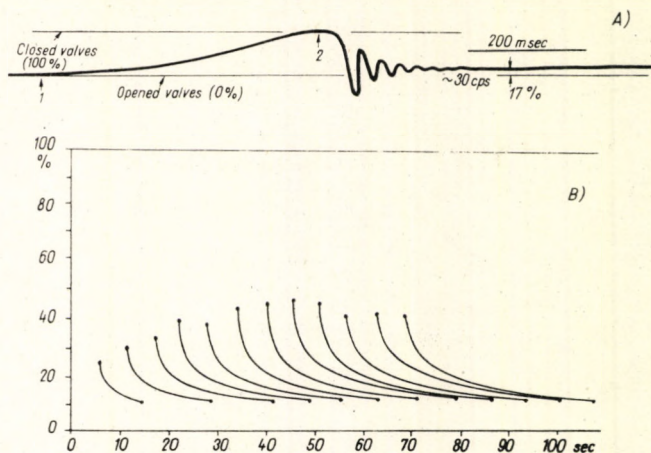


Fig. 4. A. — Oscillatory response of the system when the shell was suddenly left to move after preceding closure B — Slow part of the ligament relaxation when total closure was evoked. In each minutes lasting for 30 sec the records end approximately at the same level. The effect of the slow-responding (viscous) element is observable

When total closures were evoked lasting for different periods, relaxation became slower and slower and also the separation point was elevated as the period of closure increased.

It seems to be evident that in the response two components are present: (1) a quick and reversible or (2) a slow and spontaneously hardly reversible one.

2. How does the ligament response depend on the magnitude of load?

Taking into account that a real steady state cannot be reached even in 1 hour, the following characteristics in a strict sense are transient ones. The data were obtained after the onset: at the end of quick response (less than 1 sec), at the 30th and 600th seconds.

As the crude results are depending on the position of the Q -point (Fig. 2), on the size of animals, etc., that is why the displacement (loading curves are normalized (Fig. 5):

abscisse: Q/Q_0

ordinate: D/D_0 for 1.30 and 600 sec, where Q_0 means the weight just evoking the closure (it was determined by a linear extrapolation from positions very near to the total closure);

D_0 is the displacement on the kymograph which is equivalent with the total closure.

Q and D are the loads and displacements belonging together.

It is observable (Fig. 5A) that the curve of the quick response is running below the line of 45 degree, that is:

$$m = \frac{Q_0 D}{D_0 Q} < 1$$

For the diagram of 30 sec

$$m < 1, \text{ if } Q/Q_0 < 0.5 \text{ and}$$

$$m \cong 1, \text{ if } Q/Q_0 > 0.5$$

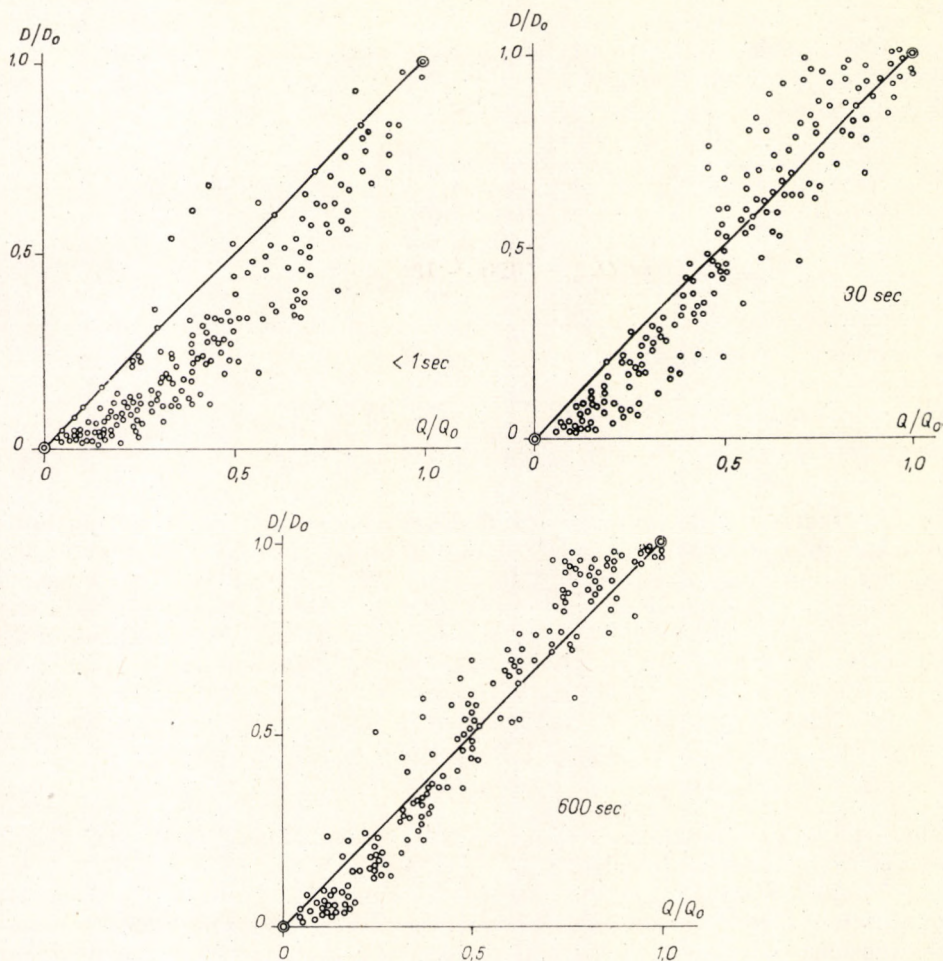


Fig. 5. Dependence of the ligament-response on the loading after 1, 30 and 600 sec from the onset. Abscisse: relative loading; ordinate: relative displacement; the 100 per cent values refer to the closure or closing weight, respectively

is characteristic. Such an S-shaped running of the latter (600 sec) curve is less explicit but for the value of m , similar statements are valid.

3. Tensions developing in the ligament and adductors during the closure of the shells

When the just closing loads, the length of lever-arms and surfaces of cross-sections are known, the above mentioned values can be easily determined.

a) The total cross-section of the two adductors was obtained by the planimetry of the attachment-plaques for the anterior (q_A) and posterior (q_P) ones, respectively:

$$q_{\text{muscle}} = q_A + q_P = 2.6 \pm 1.2 \text{ cm}^2 \text{ (mean } \pm \text{ s.e.) } (q_A = 1.5 \pm 0.9 \text{ cm}^2, \\ q_P = 2.0 \pm 0.3 \text{ cm}^2; q_A/q_P = 0.76 \pm 0.20).$$

The measured or calculated closing-loads were normalized to the "muscle-points" (Fig. 2). This point is situated between the projection of the two attachment-plaques in the median line between the two parallel tangents of the two spots, a little nearer to the posterior plaque because of the weighting with regard to the cross-sections.

The obtained values (25 preparations):

$$\begin{aligned} Q_1 &\sim 890 \pm 240 \text{ ponds} \\ Q_{30} &\sim 750 \pm 200 \text{ ponds} \\ Q_{600} &\sim 620 \pm 180 \text{ ponds} \end{aligned}$$

The maximal tensions developed in the adductors because of the ligament forces (closed position):

$$\begin{aligned} T_1 &\sim Q_1/q \text{ muscle} & 0.034 \text{ kp/cm}^2 \\ T_{30} &\sim Q_{30}/q \text{ muscle} & 0.029 \text{ kp/cm}^2 \\ T_{600} &\sim Q_{600}/q \text{ muscle} & 0.024 \text{ kp/cm}^2 \end{aligned}$$

b) When calculating the tensions in the ligament, the following must be taken into account: the position of the above defined muscle-point, its distance from the attachment of ligament (21 ± 3 mm) and the width of the line where the ligament is attached (2.0 ± 0.3 mm). For this reason the loads applied for the calculation of muscle-tension must be multiplied by a factor being at least 10. As the cross-section of ligament is 61 ± 12 mm²:

$$\begin{aligned} T_1 &\sim 1.5 \text{ kp/cm}^2 \\ T_{30} &\sim 1.25 \text{ kp/cm}^2 \\ T_{600} &\sim 1 \text{ kp/cm}^2 \end{aligned}$$

When the adductors contract these ligament tensions occur upon quick and long-lasting closures, respectively.

It can be stated, that because of their unlike positions, cross-sections and different lever-arms of force by which they influence each other, the maximal tensions of muscle are 30–50 times lesser than those of the ligament if the just closing contraction is taken into account. It is, however, widely known, that the tonically closed muscles are able to develop forces significantly higher than the calculated ones.

4. Young modulus of the elastic component of ligament

In order to calculate the Young modulus it was assumed that during the closure of shell, the elastic fibres of the ligament are elongated and the energy responsible for the opening forces is accumulated by this way. This is quite plausible because in the ligament of *Anodonta cygnea* any elastic, compressible core similar to that of the *Pecten maximus* (TRUEMAN, 1953) is not present.

To calculate the Young modulus the following quantities are necessary:

1. The length of the fibres of ligament before and after elongation: l_0 and $l_0 + \Delta l$; it is sufficient to know $\Delta l/l_0$;
2. cross-section of the ligament
3. the magnitude of the elongating force

ad 1. In *Fig. 6* the shape of shell section is demonstrated, near to the hinge-ligament and perpendicular to the attachment plane of the ligament. Two idealized positions are demonstrated: the closed and the opened ones. It is seen that the plane of shell surface at the attachment is not perpendicular,

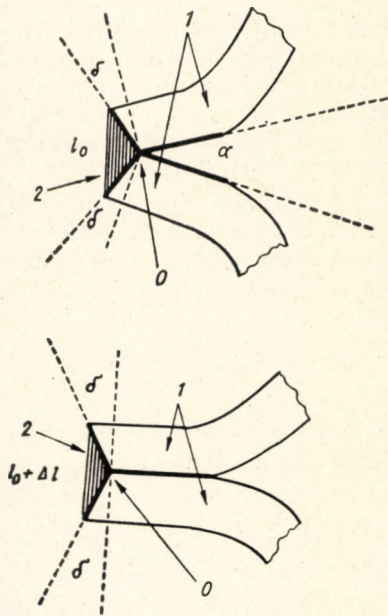


Fig. 6. A section of the shell proximally to the hinge-ligament. The plane is perpendicular to the line of ligament. Closed and opened positions. Simplified morphology (see in the text). Designations: 1 = shells; 2 = ligament; 0 = centre of rotation; δ = angle of the shell margin out of 90 degree; α = the angle of the opened shells

but inclines at a higher angle. The plus of this angle out of 90 degree (25 measurements) is:

$$\delta \sim 25 \pm 6^\circ$$

The angle of the opened shells without loading:

$$\alpha \sim 31 \pm 18^\circ$$

Observing the opened and closed positions in *Fig. 6* it can be easily concluded that the relationship among l_0 , Δl , δ , α , and d (the width of shell edge) is the following:

$$l_0 = 2d \cos \left(\delta + \frac{\alpha}{2} \right) \text{ and}$$

$$l_0 + \Delta l = 2d \cos \delta$$

The relative elongation (25 preparations):

$$\frac{\Delta l}{l_0} = \frac{\cos \delta}{\cos \left(\delta + \frac{\alpha}{2} \right)} - 1 \sim 20 \pm 15 \text{ per cent}$$

ad 2. The cross-section of the ligament was obtained by direct planimetry of the attachment surfaces:

$$q \sim 61 \pm 12 \text{ m/m}^2$$

To calculate the force attacking directly the fibres of ligament the following quantities are necessary: perpendicularly measured distances of Q -point from the line of hinge-ligament, α and δ angles. Torsions and differences of the width of attachment surface were neglected. As the mentioned distance was 63 ± 14 m/m, $d \sim 2.0 \pm 0.3$ m/m, — after taking into account α and δ as well — the measured closing-weights must be multiplied by a factor of about 20–30. The supposition, forming the basis of the calculations is an angle-lever mechanism whose assumption is plausible from *Fig. 6*. The centre of rotation is the 0-point (*Fig. 6*). Naturally this mechanism is also simplified (for example, the contact of the two shells at the hinge ligament is not point-like and perfect, etc.).

As the presence of an elastic component is manifested in the quick reversible response to loading, that is why only that Young modulus was calculated, which refers to the quick-loading values (see the quick component of *Fig. 3*). The modulus obtained by such a way (25 animals):

$$E = \frac{10}{\Delta l} \cdot \frac{F}{q} \sim 2815 \pm 1774 \frac{\text{ponds}}{\text{m/mm}^2}$$

The F is the force causing a prompt closure elongating the fibres according to the $(1_0 + \Delta l)/1_0$ ratio.

Discussion

From the results it seems to be quite clear that the force, loading the adductors is increasing with the amplitude of the muscle contraction. The relationship is not linear. Such a character of the muscle-loading speaks for the assumption that the initial value laws observable in the response of adductors stimulated indirectly (LÁBOS et al. 1970) is explained in a part by the load originating from the ligament and increasing with the increasing level of start of the contraction. According to this law the muscle responds to the same stimuli by a smaller contraction when its original steady level corresponds to a more contracted state.

A further conclusion concerning the in situ muscle function may be related to the well known (PAVLOV, 1885; SALÁNKI and LÁBOS, 1963; LÁBOS et al. 1969) relaxations. These special responses usually are explained by the presence of inhibitory innervation. But this supposed inhibition can be responsible only for a decrease in the muscle tone. The actual relaxation could be evoked by the significant forces of ligament which are able to elongate the muscle in this (inhibited, or of low mechanical resistance) condition.

On the basis of the results obtained the conclusion can be drawn that in a mechanical equivalent of the ligament at least 1 elastic and 1 viscous component must be present. However, their serial or parallel coupling is not suitable and only a mixed circuit can approximately simulate the observed phenomena. In *Fig. 7* the presented model simulates the response-type characteristic of the ligament. Such a mechanical model was applied for example by CHAPMAN (1953) to describe mechanically the mesogloea of *Calliactis parasitica* and *Metridium senile*. CHAPMAN thinks that the components of the equivalent reflects certain muscle and connective tissue components of the

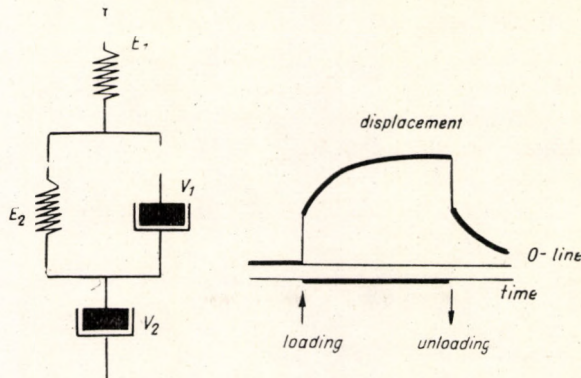


Fig. 7. A possible mechanical equivalent simulating the time behaviour of the hinge ligament

body-wall of the Coelenterates. As that in muscle physiology similar visco-elastic models are often applied (see for example PRINGLE, 1960), that is why a similar description of the adductors is needed to realistic analysis of the in situ adductor responses.

The Young modulus of the elastic component is surprizingly near to the data of TRUEMAN (1953) obtained for *Ostrea edulis* and *Lutraria lutraria*: 2000–2300 gs/mm². In these cases the elastic component is thought to be consisting of compressed and elongated elements. It is probable that in the case of *Anodonta*, the situation is similar but with a dominance of elongation-mechanism. It is interesting from this point of view that in the equivalent demonstrated in Fig. 7, two elastic components had to be supposed. However, their finer identification needs a further analysis. In general, the elastic elements are situated on the inner, the viscous ones on the outer parts of the hinge-ligament.

Appendix

In first approximation, the behaviour of the elastic component can be regarded as a linear oscillator. Because of the very high damping being present in the whole animal (muscle, visceral parts), this oscillatory behaviour has not any in situ significance, but such an analysis gives information about the properties of the elastic parts of the ligament.

At the experimental conditions, when the mass of fixed writer-lever is negligible beside that of the shell, the observed frequency of oscillating shell was:

$$F \sim 30 \pm 3 \text{ Hz}$$

(see Fig. 4A).

It is known that this frequency is determined by the mass of the shell (M) and by the spring constant of the ligament (K) in the following way:

$$F = \frac{1}{2\pi} \sqrt{\frac{K}{M}}$$

Hence

$$K = (2\pi F)^2 M = M\omega^2.$$

As F and M are determined ($M = 21 \pm 8$ g):

$$K \sim (6.28 \cdot 30)^2 \cdot 20 \sim 7.2 \cdot 10^5 \text{ dyn/cm}$$

The damping factor at the given condition may be determined from the ratio of the successive amplitudes:

$$\zeta = \frac{-1}{2\pi N} \cdot \frac{Y_n}{Y_n + N} \sim 0.1 \pm 0.05$$

where Y_n is the n -th amplitude of oscillation. From here the viscous damping coefficient (D):

$$D = 2\zeta KM = 2\zeta M\omega_0 \sim 0.20 \cdot 20 \cdot 188 \text{ g/sec} = \\ 754 \text{ g/sec} = 754 \text{ dyn} \cdot \text{cm}^{-1} \cdot \text{sec}$$

The well-known differential equation of the applied linear model:

$$My'' + Dy' + Ky = 0 \text{ or } y'' + 2\zeta\omega_0 \cdot y' + \omega_0^2 \cdot y = 0$$

With the coefficients expressed in CGS units:

$$20 y'' + 754 y' + 720.000 y = 0 \text{ or}$$

$$y'' + 38 y' + 36.000 y = 0$$

From here the transient response for step function:

$$Y_t = \frac{1 - \zeta\omega_0}{\sqrt{1 - \zeta^2}} \cos [\sqrt{1 - \zeta^2} \omega_0 t - \sin^{-1} \zeta] \sim \\ \sim e^{-19t} \cos [190t - 0.1] \sim \frac{\cos 190 t}{e^{19t}}$$

Summary

Certain, in situ mechanical properties of the hinge-ligament of *Anodonta cygnea* L. are described:

1. The ligament consists of elastic and viscous elements. A plausible equivalent is given.
2. A crude Young-modulus for the quick, elastic compound is:

$$2.8 \pm 1.8 \text{ kp/mm}^2$$

3. The tensions developing in the participating ligament and adductors during the closure of shell:
 T (muscle) 0.03–0.04 kp/cm² (minimal)
 T (ligament) 1–1.5 kp/cm² (maximal)
4. Dynamic constants determined for a linear approximation of the freely moving shell-ligament system are:
 frequency ~ 30 Hz; $K \sim 7.2 \cdot 10^5$ dyn/cm;
 damping: ~ 0.1 ; $D \sim 754$ dyn \cdot sec \cdot cm⁻¹.
5. The elastic ligament can be one of the causes of the in situ observable relaxations and it is in a part responsible for the initial value laws described for the adductor muscles (LÁBOS et al. 1970).

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ANODONTA CYGNEA L. (PELECYPODA) SAROKPÁNTSZALAGJÁNAK
NÉHÁNY MECHANIKAI JELLEMZŐJE IN SITU

Lábos Elemér

Összefoglalás

Szerző *Anodonta cygnea* L. sarokpánt-szalagjának néhány mechanikai tulajdonságát elemezte in situ.

1. A szalag elasztikus és viszkózus elemekből áll, melyek valószínű ekvivalensét megadta.

2. Az elasztikus elemek bruttó YOUNG-modulusa

$$2,8 \pm 1,8 \text{ kp/mm}^2$$

3. Záráskor a szalagban 1—1,5 kp/cm²,

az izmokban legalább 0,025—0,035 kp/cm² feszültség jön létre.

4. A szalag elasztikus elemeinek dinamikus állandói: Sajátfrekvencia ~30 Hz, rúgóállandó ~7,2 · 10⁵ dyn/cm, csillapítás ~0,1, csillapító tag ~754 dyn. sec. cm⁻¹.

5. A záróizmok in situ ernyedésének egyik okozója a rugalmas szalag.