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INITIAL VALUE LAWS APPLIED TO SPONTANEOUS RHYTHM, EVOKED OSCILLATIONS AND APERIODIC RESPONSES OF ANODONTA ADDUCTOR MUSCLES

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The law of initial value (L.I.V.) was formulated firstly by WILDER (1931) and describes correlations between the initial value of a biological parameter and its change after stimulation. It shows also that on repeating a stimulus, its effect becomes less and less; and when the system is unbalanced, its behaviour is often oscillatory (WILDER, 1962; SOLLBERGER, 1965).

It had been observed earlier, that when the cerebrovisceral-connectives (CVc) of *Anodonta* are excited, the responses of the posterior adductor muscle (PAM) are variable, even when the stimulus parameters are constant. In such cases the parameters of the response seem to be influenced by the initial muscle-length, its eventual change in a given direction, previous stimulation and the phase of the periodic activity (SALÁNKI and LÁBOS, 1963).

After stimulating the CVc, the adductor muscle in many instances, performs rhythmic contractions and approaches the initial or a new tonus level. Such homeostatic or servomechanism properties are characteristic of a system which follows the L.I.V. (SOLLBERGER, 1965). Because of this close analogy, a systematic and more exact analysis of the related phenomena was considered desirable.

Methods

Both spontaneous and evoked contractions and relaxations of the freshwater mussel's *(Anodonta cygnea* L.) posterior adductor muscle (PAM) were recorded and analysed. The animal was taken out of the water and the anterior and posterior adductors were disconnected mechanically as described earlier (SALÁNKI and LÁBOS, 1963). One of the shells was fixed while the other was connected to a lever, recording the muscle displacement on a kymograph. The muscle was loaded only by the force of the ligament ($\sim$0.5 kg) connecting the two shells.

Evoked contractions were elicited by stimulation of the cerebro-visceral connective (CVc) with square pulses. The electrodes were placed in the middle of the uninjured nerve.

A given level on the kymograph records *(Fig. 1)* represents an actual muscle-length (l). The level representing the muscle-length when the shells are closed is designated with l_0. The value l_0 is the shortest under the experi-

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mental conditions, as further contraction is prevented by the apposition of the shell margins. The level l can be measured as the deviation from l_0 that is by $(l - l_0)$.

The size of contraction (c) was measured at its maximum, while that of the relaxation (r_T) at a given time (T) after the onset of stimulation. The values of "c" are given in arbitrary units which are the same for each experiment.

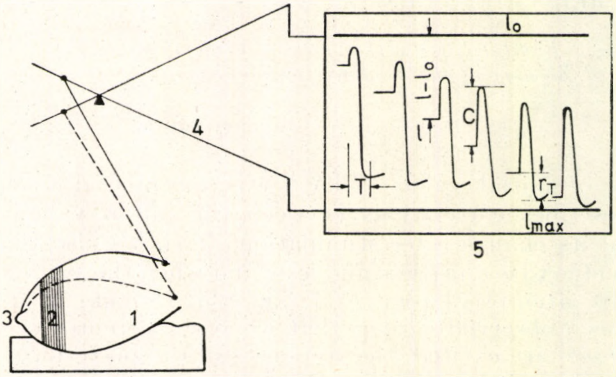

Fig. 1. Experimental arrangement and designations
1 — mussel; 2 — posterior adductor muscle (PAM); 3 — ligament; 4 — lever recording displacements; 5 — records; l_0 = muscle length at closure of the shells; l_{max} = maximal length of PAM; l = an actual muscle length; c = amplitude of contraction; T = time after stimulus-onset; r_T = effective relaxation below initial level at T moment

Results

1. The dependence of the amplitude of evoked contractions on the initial length of the muscle

As the PAM is a mixed tonic and phasic muscle, it can work at different lengths and after spontaneous rhythmic contractions is able to return to the actual working level. The contractions represent an increase in the muscle tension, as at shorter muscle length the load is represented by the increased tension of the ligament, which is higher. For this reason both steady state muscle length and tension vary from animal to animal, as do the temporal relations as well. Their levels influence the size and time course of the spontaneous or evoked phasic contractions and relaxations.

On stimulating the CVc by uniform trains of pulses, the evoked contraction is smaller if the initial level of tonus is higher i.e. when the initial muscle length is shorter. This correlation is always close. In *Fig. 2* the typical negative linear correlation is demonstrated by line A. Its linearity is equivalent to the following law: the steady state amplitude (c) of the evoked contraction is always a constant ratio of the maximal response that could be elicited at the given muscle length. In the demonstrated case (A on *Fig. 2)* the slope-parameter of the regression line is 0.53. In short experiments this ratio (k) is constant for a given animal, but it deviates with different animals and depends on the parameters of the stimulus. The average of k calculated from data of 20 animals was 0.50 ± 0.19 (mean ± s.d.) being 0.10 and 0.95 the extreme values. The stimulus-parameters were: 4 msec, 8 cps, 20 V, 60 sec.

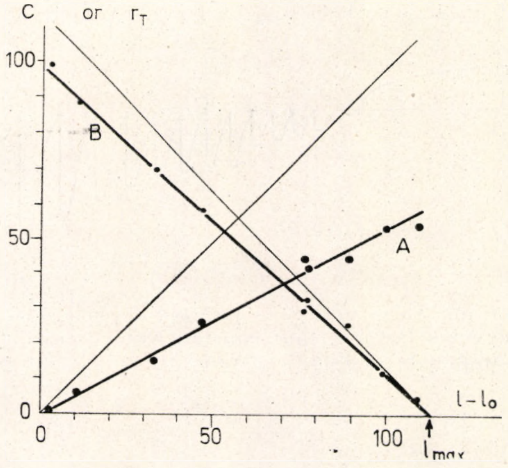

Fig. 2. Conctractions (c) and relaxations (r_T) are plotted against initial level $(l-l_0)$

$$A - c \sim 0.53 \cdot (l - l_0);\ B - r_T \sim -0.88 \cdot (l - l_0)$$

Coefficients of correlation are near to ± 1, resp. The origin means l_0 and the abscisse is intersected by r_T line at l_{max}. The two further lines follow slopes of ± 1 (45°) and represent the maximal possible contractions and relaxations in the actual case. Parameters of stimulus-train: 20 volts, 4 msec, 8 cps, 60 sec

2. *The dependence of the amount of relaxation following evoked contractions, on initial muscle-length*

In cases when the relaxation period was not interrupted by spontaneous contractions a clear dependence of the size of relaxation on the initial muscle length was found (B-line in *Fig. 2)*. The size of relaxation increases with increasing initial tonus level, that is with decreasing initial length of the muscle. This linearity represents a similar law as that was described for the contractions, however the linearity of this dependence is not always so strict as that of the contraction-response.

3. *The differences in the magnitude of contraction evoked at a constant length of the muscle*

When the CVc is repeatedly stimulated at a constant muscle length, the evoked contractions are still not uniform. *Fig. 3* shows that the amplitudes of the successive responses elicited by identical trains of pulse at a medium level of length (or tonus) decrease successively. The steady state magnitude of the n-th response (c_n) is proportional to that of the first one (c_0) and nearly exponentially decreases with the sequence number of repeated stimuli *(Fig. 4A)*.

The time-intervals between the successive contractions in *Fig. 3* are determined by the returning to the initial muscle length. If these intervals (τ_n) were measured and plotted against the amplitude of the following contractions (c_{n+1}) a correlation with a linear section was found *(Fig. 4B)*.

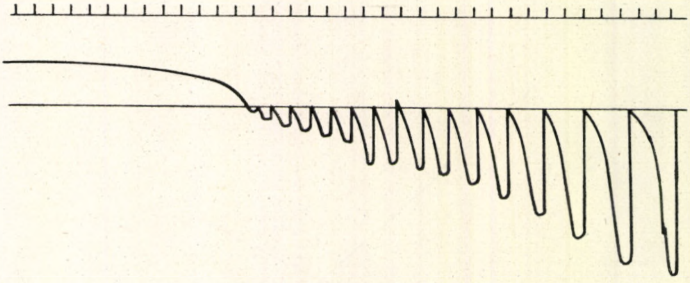

Fig. 3. Responses of PAM. The stimuli to CVc were applied at the moments when the length of relaxing muscle reached a certain, constant value (horizontal line on the figure). Time-scale 60 sec. Parameters of stimulus-train. 10 volts, 4 msec, 8 cps, 30 sec

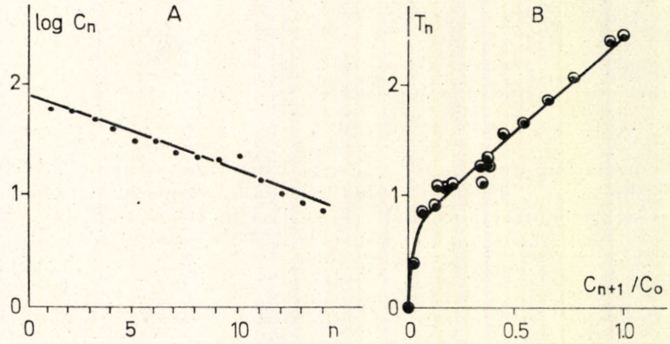

Fig. 4. A — Logarithms of amplitudes of evoked contractions ($\log c_n$) are plotted against the sequence number (n) of successive stimulus trains. Data are taken from Fig. 3. The regression-line represents an equations $c_n \sim c_0 \cdot \exp(-0.16 \cdot n)$. Coefficient of correlation $r \sim 0.9$. — B Recurring times (τ_n) are plotted against the relative amplitude of n + 1-th response (c_{n+1}). Approximation of linear part:

$$\tau_n \sim 1.95 \cdot \frac{c_{n+1}}{c_0} + 0.68 \text{ (min)}$$

4. *Oscillatory after-effect*

In general the prepared PAM may contract rhythmically with very different average frequencies (5—60 cph); and on stimulating the CVv, its evoked response consists of contraction and relaxation, often below the initial level. Depending on the stimulus strength and the initial muscle length, the PAM may become more or less relaxed, than it was before stimulation.

In a number of instances the evoked response is followed by a series of rhythmic contractions; whose frequency and amplitude is higher than during the control period. The time course of these oscillatory after-effects is variable. The tonus may return to the original or to a new steady level finally *(Fig. 5a—j).*

The trend of the change in tonus depends on the ratio of the amplitudes of contractions and subsequent relaxations and on the frequency of after-oscillation as well. When relaxations are less than the contractions *(Fig.*

5c, h) or absent *(Fig. 5d)* the tonus increases. The high frequency of the rhythm is favourable to a higher tonus because the contractions following each other in short intervals interrupt the actual relaxation *(Fig. 5e, g)*. The evoked oscillation starts usually after a latent period. Its frequency decreases, but sometimes in a short initial period a slight increase is observable *(Fig. 5a, h, i)*.

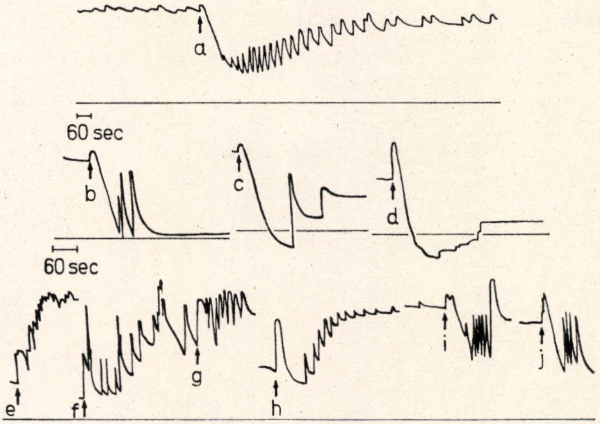

Fig. 5. Different cases of evoked after-oscillations taken from three different animals (a; b—d; 4—j). Parameters of stimuli to CVc: 10 V, 4 msec, 8 cps, 20 sec. The onset of stimulations is marked by arrows. Horizontal lines show the same length of muscle. Time-scale of b is valid for c—j as well

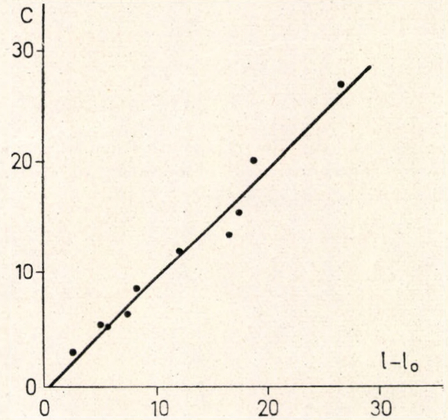

Fig. 6. Correlation between the size of phasic, evoked after-contractions (c) and the level of muscle length $(l - l_0)$ from that they were starting. The regression line is $c \sim 0.94 \cdot \cdot (l - l_0)$. Coefficient of correlation $r > 0.9$

The amplitudes of spontaneous oscillatory contractions bear a relation to the tonus level from which they start. The higher the initial level, the smaller the amplitude. The function is fairly linear at most values of the initial tonus *(Fig. 6)*. In the cases analyzed in detail (8 animals) the constant of L.I.V. was $k = 0.68 \pm 0.11$ (mean ± s.d.; 0.28 — 1.00).

The first few contractions (and its k-value) sometimes do not decrease notwithstanding the increasing initial level *(Fig. 6a)*.

The oscillatory after-effect evoked by repeated stimulation becomes, step by step, less oscillatory *(Fig. 7)*. In this case the decreasing number of contractions after four successive stimulations were 17—7—3—0. In general

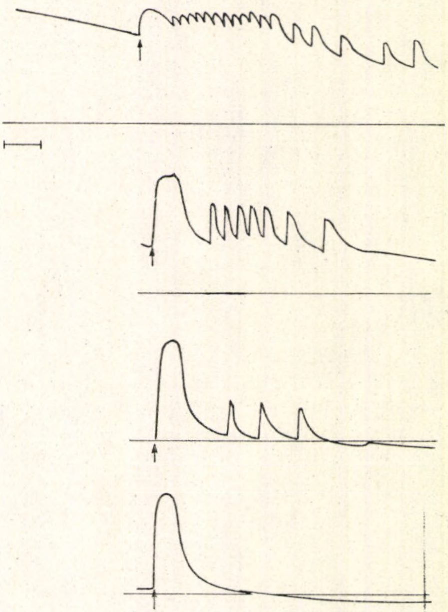

Fig. 7. Adaptivity of after-oscillation. The repeated stimulation abolish the after-effect· The horizontal lines show the reference-level. The onset of stimulation marked arrows· Parameters of stimulus-trains: 20 V, 4 msec, 8 cps, 60 sec. Time mark: 60 sec

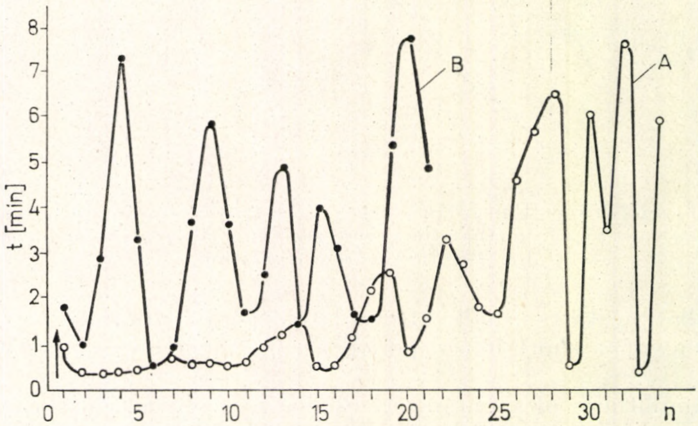

Fig. 8. The length of successive time intervals between the evoked after-oscillations are plotted against their sequence-number. A — CVc is intact; B — The same plot but after cutting the CVc After A an interval of about 30 min was left to pass. Parameters of stimulations: 10 V, 4 msec, 8 cps, 60 sec

after a few stimuli the oscillations are abolished. It is possible to subsequently elicit a steplike increase of tonus by mechanically stimulating the syphon. The previously abolished oscillation can be seen yet again.

The frequency of after-oscillations changes considerably when the connection of CVc with the cerebral ganglia is cut. In Fig. 8 the successive intervals between the rhythmic contractions of after-oscillation are plotted against their sequence number. *Fig. 8A* shows the control, where the decrease of frequency is well shown. Immediately after cutting the CVc, the cut end was stimulated by the same stimulus used in the control. The intervals between the evoked oscillatory after-contractions are demonstrated in *Fig. 8B*. It can be seen, that the frequency is decreased and its trend is less than in the control.

5. *L.I.V. for spontaneous contractions at the end of the active period*

The adductor muscles of Anodonta undergo alternating periods of activity and inactivity. During activity the muscles are partially relaxed and rhythmic contractions are observed. Towards the end of an active period characteristic contractions appear. These manifest themselves as adductions of decreasing amplitude. It was found that the amplitude decreases with the decreasing initial legth of muscle. This correlation proved to be linear except for contractions starting at very high tonus levels *(Fig. 9A)*. In the example shown the constant of L.I.V. is 0.58.

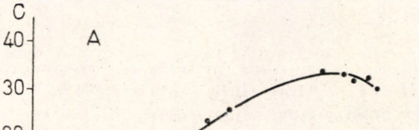

Fig. 9. The amplitude of contractions (c) are plotted against the initial muscle length $(l - l_0)$. L.I.V. for A — spontaneous catch-contractions; B — evoked aperiodic response; C — after-oscillations

The described linearities in sections 1, 4 and 5 are not valid at extremely high or low initial levels, where a decrease of the constant k was found *(Fig. 9A)*. Similar non-linearity occurred also in case of evoked contractions *(Fig. 9B)* and oscillatory after-effect *(Fig. 9C)* at extreme initial values.

When contractions of a whole active period were analyzed, a more complex phenomenon was found. The plot of amplitudes of consecutive contractions (c) against the initial muscle length $(l - l_0)$ does not follow a single line but describe a loop *(Fig. 10)*, which can not be characterized by a single k value. The arrows starting from and returning to the point corresponding to the shortest muscle length approximately follows the consecutive contractions. Some contractions are out of the main loop but they seem to form organized pattern.

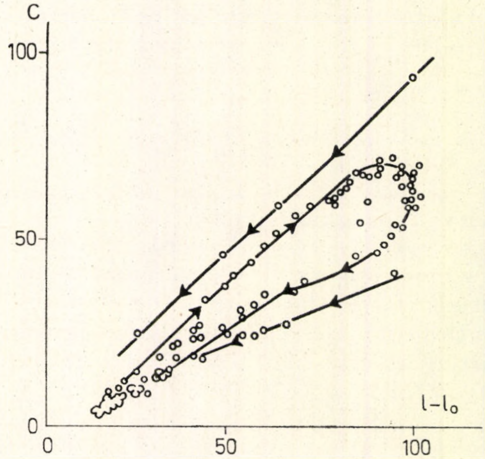

Fig. 10. Plot of all phasic contraction-amplitudes (c) observed in a whole active period of an animal against the level at that they start $(l - l_0)$. The linear parts represent a L.I.V. with constant k. It is observed that during an active period an inhomogeneous cycle of L.I.V. — variation takes place

Discussion

The L.I.V. applied here for evoked aperiodic or oscillating phasic contractions, for the spontaneous closing contractions and for relaxations can be written in a general form:

$$\Delta l \sim k \cdot (l - l_0)$$

when l is not extreme in value.

Concerning the origin of this law the following can be stated. In situ the adductors are stretched by the force of the elastic ligament connecting the two shells. When the shells are more closed by the shortening of the muscle, this force is greater. A given extension of the ligament can be evoked by a force being nearly proportional to its deformation (extension and/or compression; unpublished). Therefore a tension-increase in the muscle proportional to the magnitude of contraction must be produced during the shortening. Thus the L.I.V. is equivalent to a length-active tension or to a load-active tension diagram. In such a way the muscle is not working isotonically but against a nearly linearly increasing load.

A source of the observed differences in the responses of the adductors when stimulating at different initial length with identical stimuli (Salánki and Lábos, 1963) has been found mainly in the above described length-tension-correlation.

The L.I.V. for artifical stimulation is seen to be valid when applying the same stimulus. As the same law is valid for either spontaneous or evoked oscillatory, free running contractions, a conclusion is suggested that these actions must be generated by the excitation of same magnitude of the innervating centres. On the other hand, when the contractions of the same muscle working spontaneously cannot be described by a single k value *(Fig. 10)*, different level of nervous excitation must be supposed. In this manner the k of L.I.V. can give information about the excitation running out from ganglia towards the muscle.

The L.I.V. of relaxation can be interpreted also by taking into account the tension of ligament. At lower initial muscle-length, when the loading force of ligament is higher, the relaxation under the initial level is proportionally higher *(Fig. 2B)*. Nevertheless by this explanation the differences in the final relaxation level can not understood. For this reason during the relaxation-process intrinsic changes of the mechanical properties of muscle must be taken into consideration as well.

Phenomena demonstrated in *Fig. 3* and *4* clearly show that the explored L.I.V. is not valid when the stimulation is too frequent. In such a case a decrease of k value was observed. It could be considered as a result of the accumulative effect of previous stimuli and/or as a dependency on the initial speed of relaxation.

Systems studied by basimetry show all the phenomena observed in the behavior of *Anodonta* adductor:

1. law of initial value *(Fig. 2, 9)*
2. law of initial speed *(Fig. 3)*
3. oscillatory behavior after disturbing *(Fig. 5)*
4. adaptivity of oscillatory behaviour and of the evoked contractions *(Fig. 7* and *3)*.

It seems to be reasonable that such properties are concerned with an automatic, adaptive control (servomechanism) of adductor behavior. The sources of the phenomena or distribution of parameters in the components (muscles, ganglia, synapses, ligament, receptors) of the whole system are not known exactly. It is very probable that different functional feed-back loops are responsible for the whole behavior. A suitable and quantitative description or modelling of the behavior must be carried out in terms of automatic control. Approximations given by an application of basimetry are less general and purely phenomenological but it gives a unified explanation and may form a first step in a cybernetic evaluation of the regulation of adductors.

Summary

Initial value laws were demonstrated as being valid for spontaneous rhythmic contractions, and evoked responses (contraction and relaxation) of *Anodonta* adductor muscle.

The following group of phenomena has been observed and analyzed

1. quasi-linear relationship between responses and initial values,
2. homeostatic after-oscillations,
3. adaptive behavior of evoked contractions and that of after-oscillations.

The idea of basimetry seems to be useful in explaining some of the phenomena observed, however it does not provide a complete explanation. Conclusions may be drawn about the roles of innervation, the muscle itself and the ligament, in the bahavior of the adductors.

Acknowledgements

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KEZDETI ÉRTÉKTÖRVÉNYEK ALKALMAZÁSA ANODONTA ZÁRÓIZOM SPONTÁN ÉS KIVÁLTOTT OSZCILLÁCIÓIRA, VALAMINT APERIODIKUS VÁLASZAIRA

Lábos E., B. Glaisner és *J. Salánki*

Összefoglalás

Kezdeti értéktörvények érvényességét demonstrálták *Anodonta* záróizom spontán ritmikus válaszaira, valamint kiváltott oszcillációira (kontrakció és ernyedés).

Az alábbi jelenségcsoportokat vizsgálták és analizálták:

1. a válasz és a kezdeti érték közötti kvázi-lineáris viszony;
2. homeosztatikus utóoszcilláció;
3. a kiváltott kontrakció és utóoszcilláció adaptív jellege.

A bazimetria alapelvei használhatónak bizonyultak a vizsgált jelenségek magyarázatában, azonban elégségesnek nem tekinthetők.

Az eredmények alapján következtetések vonhatók le az innervációra, valamint az adduktorok működésében szerepet játszó izom és ligamentum sajátosságaira vonatkozóan.

ПРИМЕНЕНИЕ НАЧАЛЬНЫХ КОЛИЧЕСТВЕННЫХ ЗАКОНОВ ДЛЯ СПОНТАННОЙ И ВЫЗВАННОЙ ОСЦИЛЯЦИИ И АПЕРИОДИЧЕСКИХ ОТВЕТОВ АДДУКТОРА АНОДОНТЫ

Э. Лабош, Б. Глезнер и *Я. Шаланки*

Проверяли приложимость начальных количественных законов к спонтанным ритмическим ответам и вызванным осцилляциям аддуктора Анодонты (сокращение и расслабление).

Исследовались и анализировались следующее группы явлений:

1. Квази-линейное отношение между ответом и начальной величиной,
2. гомеостатическая пост-осцилляция,
3. адаптивный характер вызванного сокращения и пост-осцилляции.

Основные принципы базиметрии оказались пригодными для объяснения исследованных явлений, в то же время нельзя их рассматривать достаточным. На основе данных можно сделать выводы об иннервации, и о свойствах лигамента и мышцы, важных для работы аддукторов.