RELATION BETWEEN THE MECHANICAL PROPERTIES OF MUSCLES AND THEIR STRUCTURE ON THE MOLECULAR LEVEL

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Mechanical properties of the vertebrate skeletal muscles are satisfactorily described on the basis of a new model of the myosin molecule packing into the thick filament.

1. INTRODUCTION

It is well known that muscle contraction is a result of the relative movement of the interdigitating filaments: thick, containing myosin, and thin, containing actin [1, 2, 3]. Recent studies (for review, see [3, 4, 5]) indicate that the interaction between myosin heads and actin filaments, coupled with ATP hydrolysis and regulated by Ca^{2+}, is a decisive event in muscle contraction. Moreover, it is well established that the actin-myosin interaction is highly stereospecific. That means that during contraction the cross-bridges, which cyclically protrude from the thick filament backbone towards surrounding thin filaments, have to provide into close contact thousands of the specific binding-sites on actin monomers and myosin heads. The cross-bridge movement is conditioned, of course, by a manner of packing of the myosin molecules into the thick filaments and the actin monomers into the thin filaments [6]. On the other side, force generated during muscle contraction depends on the way of the cross-bridge movement. So, the mechanical properties of the muscles necessary to understand the muscle structure on the molecular level should be taken into consideration.

Structure of the myosin molecule is fairly complex [7, 8]. It consists of two heads linked by the long tail. Up to now, several models of the thick filament

structure for vertebrate skeletal muscles have been proposed (for review, see [9, 10]). The models have certain features in common: the straight tails of myosin molecules lie parallel or near to the filament axis (see Fig. 1). Such a packing implies that the cross-bridges work in an oar-like manner, i.e., by tilting of myosin heads relative to the tail [11], by bending of the tail in addition to the head tilting [1, 2], or by bending of the head itself in addition to the foregoing [12]. The oar-like movements of the cross-bridges in the gap between the myofilaments seem to be, however, unreliable. First of all, as was previously discussed [6], this can be accounted for by a geometrical discrepancy: the length of the head is 16 − 19 nm [7], while the distance between the thick and thin filaments, at which the myofilaments can slide, may vary in the range of 9−21 nm [13, 14]. Moreover, in our opinion, the cross-bridge movement resulting from the models proposed so far yields too many degrees of freedom, what makes impossible matching between the specific binding sites required for the actin-myosin interaction. To overcome similar and other inconsistencies, we have proposed an alternative model for the vertebrate striated muscle thick filament [6, 15] and for the muscle contraction mechanism [16].

The main differences between our model and that proposed so far consist in two features. First, we have introduced the arrangement of the myosin tails along a helical track instead of the straight tails running nearly parallel to the filament axis (compare Fig. 1c with Fig. 2c). Second, instead of the symmetrical alignment of three cross-bridges in a crown, we have proposed an asymmetrical configuration (compare Fig. 1b with Fig. 2b). The new model provides not only the required geometrical accuracy, it also helps to better understand a number of mechanical properties of the muscles.
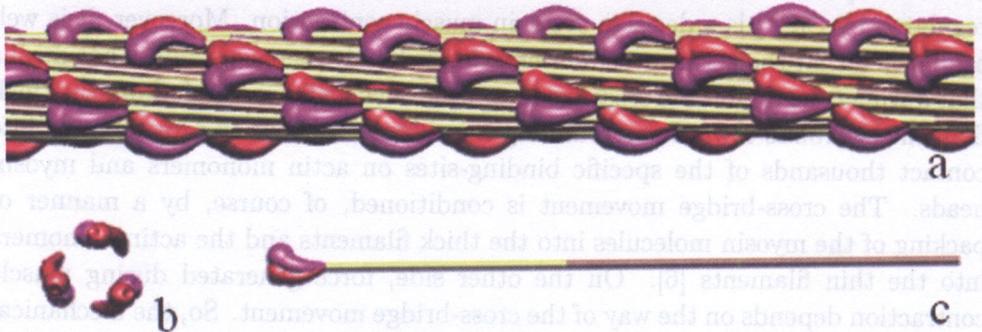
FIG. 1. Thick filament (a) with the symmetrical crowns (b) constructed from the straightening myosin molecules (c). Figs. b) and c) are presented in smaller scale than the Fig. a). Red and magenta elements represent two myosin heads; yellow and brown elements represent, respectively, the subfragment-2 and the light meromyosin of the myosin tail.
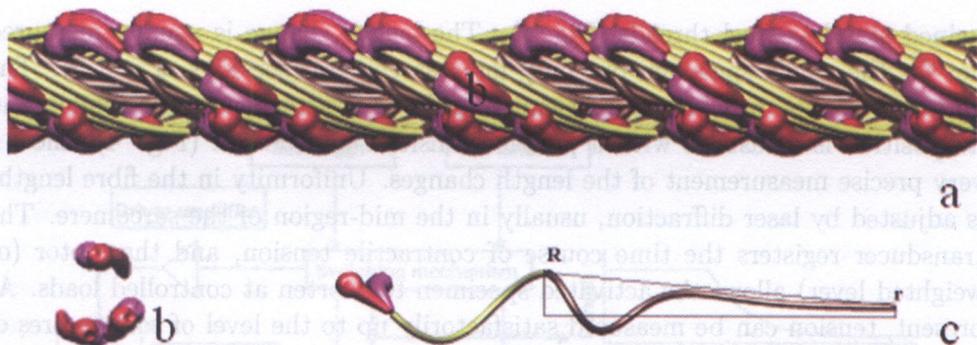
FIG. 2. Thick filament (a) with the asymmetrical crowns (b) constructed from the myosin molecules coiled along a helical track (c). Figs. b) and c) are presented in smaller scale than the Fig. a). Red and magenta elements represent two myosin heads; yellow and brown elements represent, respectively, the subfragment-2 and the light meromyosin of the myosin tail.

2. EXPERIMENTAL CONDITIONS USUALLY USED FOR MUSCLE EXAMINATION

Since the 18th Century it has been well known, that a muscle, stimulated by an electrical current, shortens. Galvani (1737–1798) was the first to demonstrate this phenomenon, and afterwards, Volta (1745–1827) explained it in terms of electrical stimulation of the muscle contraction. The stimulation consists in a depolarisation of the outer membrane of muscle fibre triggered off by an external electrical signal or by a motor nerve. The individual muscle fibres have, as was firstly established by LUCAS [17], an all-or-none character of responds to a signal of definite voltage. So, a super strong electrical stimulation would not necessarily cause a maximal contraction.

Muscle fibre along with the stimulated nerve is called the motor unit. Each nerve can activate, however, a widely dispersed fraction of the muscle's fibres. So, the higher is the required strength, the larger will be the number of motor units activated; through such a mechanism, the expenditure of chemical energy can be regulated according to the demand. The nervous system also regulates contractile strength within the motor unit itself: the higher is the stimulus frequency delivered, the higher will be the tension generated by the motor unit. The extent of contraction, in turn, depends on both the electrical property and internal ultrastructure of the muscle.

Mechanical properties of muscles are usually examined on bundles of fibres or on bundles of several myofibrils (Fig. 3). The specimen is placed in a chamber perfused with the Ringer's solution, often at 4° C. To achieve a uniform stimulation, the muscle or fibre is placed horizontally on a grid formed by alternating-polarity platinum wires. A tendon (or a bone) of the specimen is usually fixed to a tension transducer and its other end is connected to both a solenoid and a weight with

twined stainless-steel threads (Fig. 4). The length change is usually measured not for the whole specimen but only for a sarcomere (see Figs. 3 and 5). The optical methods, in which a light-emitting diode is attached to the tendon and its position is measured with a position-sensitive photodiode (Fig. 4), allow a very precise measurement of the length changes. Uniformity in the fibre lengths is adjusted by laser diffraction, usually in the mid-region of the sarcomere. The transducer registers the time course of contractile tension, and the motor (or weighted lever) allows the activated specimen to shorten at controlled loads. At present, tension can be measured satisfactorily up to the level of some scores of milinewtons (mN) and a fibril shortening in ~ 10 μsec [18, 19, 20].
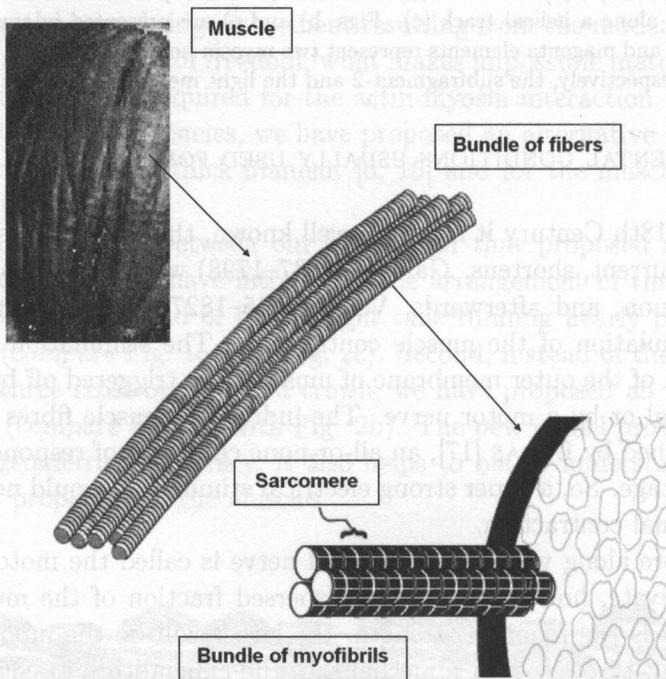
FIG. 3. Structure of the striated muscles.

Tension generated in the muscle due to the stimulation rises with some delay because the contraction proceeds in a series of events: the nerve-action potential has to cross the junction between the motor nerve and the muscle fibre membrane to depolarise the membrane; the potential created has to pass the triad to activate it; the calcium ions have to be released from the sarcotubular reticulum to diffuse onto the myofilaments the contractile proteins to be switched on. This time delay, called the latent period, is typically 10 – 15 ms after muscle stimulation.
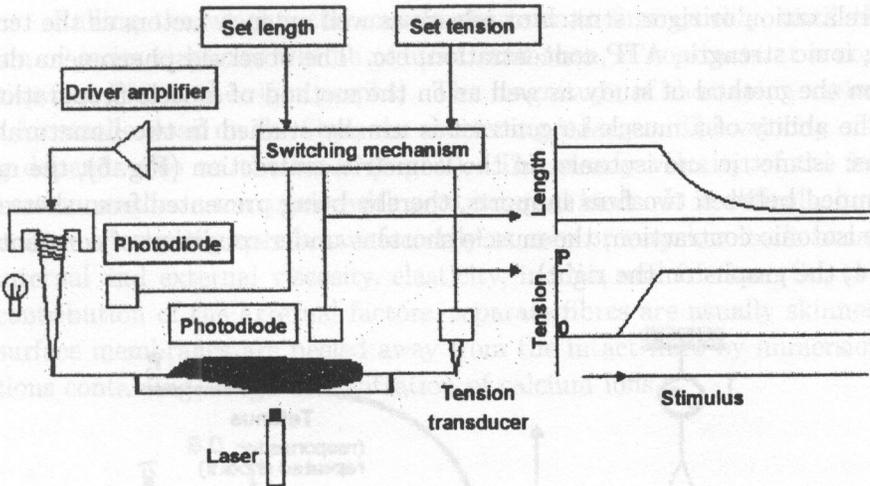
FIG. 4. General scheme usually used for examination of the muscle mechanical properties.
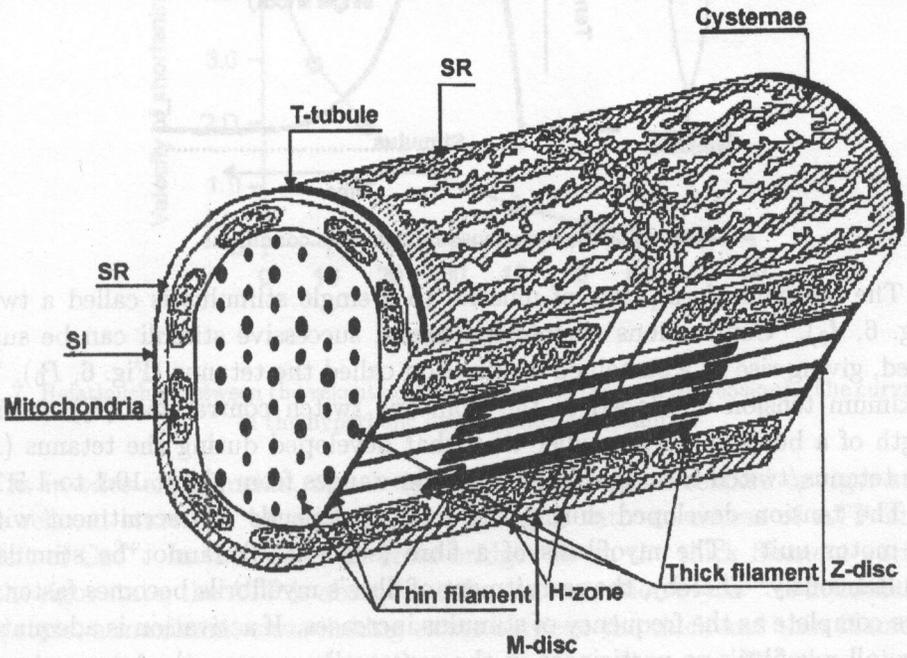
FIG. 5. Scheme of the sarcomere of the skeletal muscle; SR – sarcotubular reticulum; Sl – sarcolemma.

3. INVESTIGATED STATES OF MUSCLES

Mechanical properties of muscles depend on their states like rest or contraction, relaxation or rigor, stretch or release as well on such factors as the temperature, ionic strength, ATP concentration, etc. The observed phenomena depend also on the method of study as well as on the method of muscle preparation.

The ability of a muscle to contract is usually studied in two unnatural conditions: isometric and isotonic. In the isometric contraction (Fig. 6), the muscle is clamped between two firm supports, thereby being prevented from shortening; in the isotonic contraction, the muscle shortens under condition of constant load (Fig. 4, the graphs on the right).
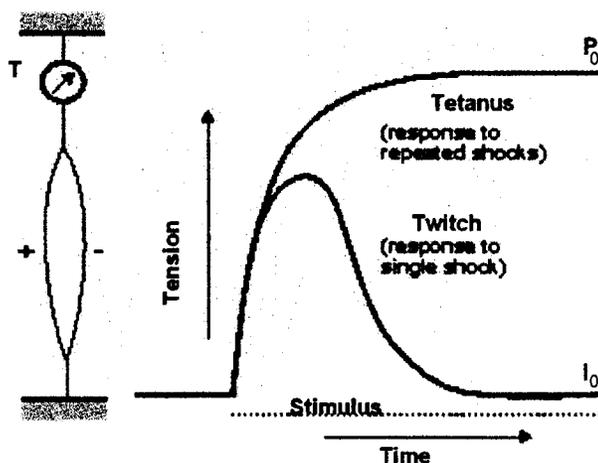
FIG. 6. Muscle response in isometric conditions.

The mechanical response of muscles to a single stimulus is called a twitch (Fig. 6, I_0). Contractions elicited by several successive stimuli can be superposed, giving rise to a sustained contraction called the tetanus (Fig. 6, P_0). The maximum tension developed in the isometric twitch contraction (I_0) at mean length of a body is much smaller than that developed during the tetanus (P_0). The tetanus/twitch ratio in different muscles ranges from about 10:1 to 1.5:1.

The tension developed during the tetanus depends on recruitment within the motor unit. The myofibrils of a fibre (see Fig. 3) cannot be stimulated simultaneously. Thereby, the recruitment of fibre's myofibrils becomes faster and more complete as the frequency of stimulus increases. If activation is adequate to allow all myofibrils to participate in the contractile process, the tetanus tension rises to maximum. In the high-frequency tetanus, the force produced by the muscle wavers even under the constant frequency of stimulation. This effect, called muscle fatigue, involves several different mechanisms.

Muscle is usually stimulated until isometric tetanic tension becomes steady. After that, the solenoid is started to slacken the thread connected to it. When the fibre tension rises to the level of the weight loading the muscle, shortening begins. Pulling the weight, the muscle shortens at first quickly, until the extension of series elasticity will be reduced, and then, it contracts at a constant velocity (see Fig. 4, the right graphs). The magnitude of shortening varies with load: for smaller load on the fibre, the shortening is larger. The velocity of shortening depends also on the loads (Fig. 7). The steady velocity of the isotonic contraction reaches a maximum with zero external loads. It is difficult, however, to measure shortening speeds satisfactorily at zero or very low loads because of the internal and external viscosity, elasticity, inertia, and friction. So, to avoid the contribution of the external factors, separate fibres are usually skinned, i.e., the surface membranes are peeled away from the intact fibre by immersion into solutions containing a high concentration of calcium ions.
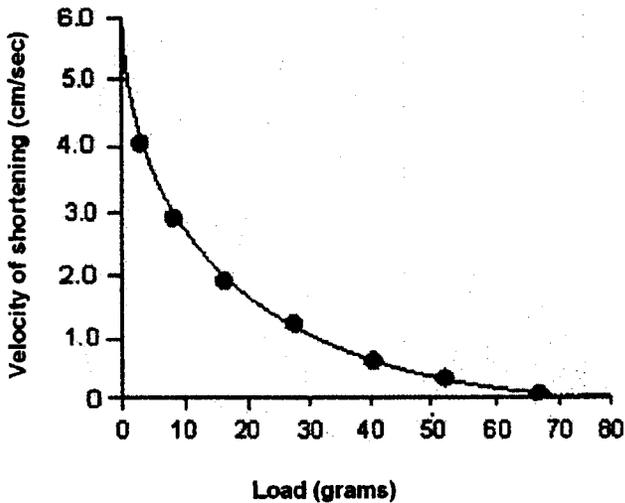
FIG. 7. Relationship between the velocity of shortening and the load (or tension). The curvature of the hyperbolic line depends on a sample.

In *in vitro* experiments, muscle is often studied under relaxed or rigor state. The relaxed state can be achieved in a solution with an excess of ATP and a deficit of Ca^{2+}. In this state, all cross-bridges lie on the thick filament surface. In the rigor state, the ATP is absent but the concentration of Ca^{2+} is maximal. In this state, a maximum cross-linking exists between the thick and thin filaments. The electron density maps of axially projected myofilaments [21] show that in the rigor muscles almost all myosin heads are arranged in the vicinity of thin filaments. This state is often considered as a final phase of contraction because all myosin heads are attached to the thin filaments.

Summarising, it seems reasonable to emphasize that in *in vitro* experiments muscles are subjected to extreme biomechanical (isometric or isotonic contraction) and biochemical (relaxed or rigor state) conditions. That leads to some indeterminacy: the tension and shortening speed (or length) cannot be evaluated at the same time; they are measured in different experiments: maximum tension is observed when there is no motion, and contraction with a maximum velocity occurs when there is no load. In consequence, some unreasonable conclusions are often drawn, for instance, that no external work is performed by muscle when it contracts with a maximum velocity, or that it exerts maximum tension continuously utilizing the chemical energy. In terms of the indeterminacy, unreasonable seems to be also evaluation of the power output (PV).

4. VISCOELASTIC PROPERTIES OF MUCLES

Viscoelastic properties of muscles have primarily been determined on the basis of the tension-length or velocity-load relationships. However, it should be borne in mind that those curves result from interconnection of the data points, each of which features a separate contraction and often depicting the values obtained from different specimens.

Muscle fibres, subjected to stretch, reveal an internal resistance in both states of the rest (resting tension) and under stimulation (passive tension). Inversely, as the muscle fibres are released from an extended length, they retract toward their natural length. During tetanic stimulation, the muscle offers a very much larger resistance than in the resting state. Unstimulated muscle offers about 0.1 per cent of the tetanic tension, and does not increase further as the stretch is continued [22]. The starting point for onset of the resting tension, relative to body length, is a major variable for different muscles, as are both the tension and relative length at which breakage occurs. The resting tension might arise from such longitudinally oriented physical structures as elastic stroma and sarcoplasmic reticulum surrounding the myofibrils, sarcolemma, connective tissues surrounding the fibre, as well as I-bridges. The I-bridges could span the thin filaments as well as the connecting filaments, or could link thin and connecting filaments [23, 24, 25]. To exclude the foregoing contributions, the tension is usually evaluated for a sarcomere from a mid-region of the skinned fibre.

In the active state, the tension-length relation measured for the bundles of fibres or myofibrils has a similar basic shape for all cross-striated muscles, especially in conditions where all myofibrils participate in the contractile process: an arching rise reaching a flat segment, then the total tension falls off, and finally rises again. Such a relation for a fibre is always flat, but that evaluated for a par-

ticular sarcomere depends on the experimental conditions. It is worth to notice that holding the fibre isometric does not necessarily mean that the sarcomeres remain isometric; the sarcomeres are naturally shorter near the ends of the fibre, and these shorter sarcomeres contract at the expense of the others [26].

To maintain sarcomeres from a mid-region of the fibre at constant length by either shortening or stretching the fibre, the servo-control is usually used. In the absolutely isometric conditions (performed for the first time by GORDON and co-workers [27]), the tension-length relation for sarcomere is broken (Fig. 9): the generated tension rises rapidly at short sarcomere lengths (up to 1.65 μm), rises less rapidly at intermediate lengths (in the range of 1.65 − 2.0 μm), is constant (between 2.0 and 2.2 μm), and falls off linearly at extended lengths (up to 3.6 μm). This result is interpreted according to the cross-bridge theory by the proportional relation between the force generated in the sarcomere and the number of cross-bridges involved between the thick and thin filaments during muscle contraction. The later investigations [28] have shown, however, that the tension-length curve can also be flat for sarcomere, which was allowed to shorten by a few percent of their initial length and then remained isometric (Fig. 8 and the dashed line in Fig. 9).
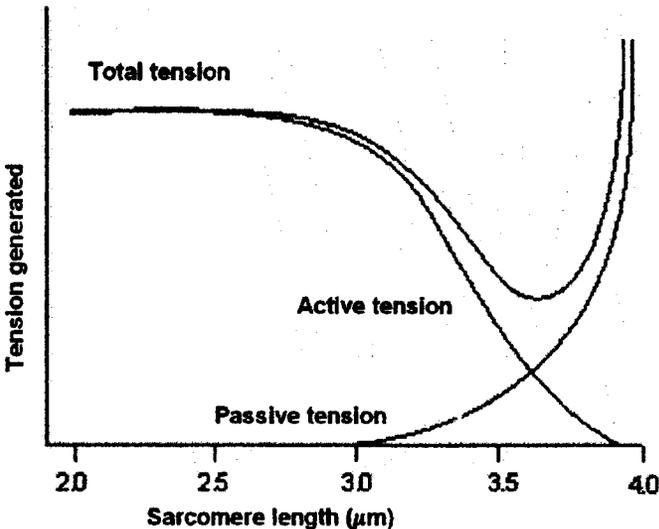
FIG. 8. Three kinds of the tension generated in a sarcomere.

Complex behaviour of the total tension at the extended lengths of the sarcomere (Fig. 8) is due to the passive tension. The passive tension starts to be measurable at the same sarcomere length for all striated muscles (Fig. 8). This specific point corresponds to the sarcomere length at which the overlap-

ping between the thick and thin filaments does not exist (Fig. 9); then, as the overlapping increases, the passive tension increases very rapidly. If the passive-tension curve is subtracted from the total one, the so-called active tension (see Fig. 8) can be obtained. Such a curve is bell-shaped for all vertebrate skeletal muscle sarcomeres. This feature might suggest that muscle sarcomere manifests the elastic properties only at the lengths larger than that corresponding to the range of active shortening, i.e., larger than 3 μm. But the so-called transient experiments have shown that the muscles reveal the elastic properties at every sarcomere length [29, 30].
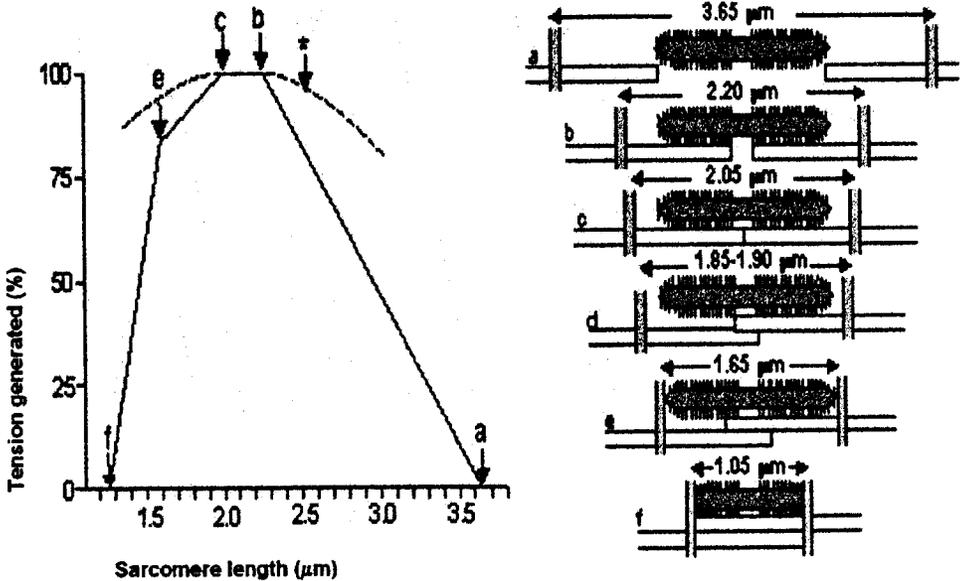
FIG. 9. Active tension generated in a sarcomere under isometric conditions with servo-control (continuous line) and without (dashed line*). The right plot shows degree of overlapping between the myofilaments.

Velocity/force (or load) relationship for muscles (see Fig. 7) differs from that for a purely elastic body. In a purely elastic body, the force depends on the magnitude of displacement relative to the equilibrium state. The force produced by a contracting muscle ($F = P$) varies with the speed of shortening, V, decreasing at higher velocities along a hyperbolic curve. The deviation from linear relationship has been described by a "characteristic equation" [31]:

$$(P + a)(V + b) = \text{constant}.$$

For suitable values of a and b, specific for the particular muscle under investigation, the theoretical relationship very closely fits that experimentally observed.

In terms of the P/V curve, the stimulated muscle manifests not only the elasticity but the viscosity too.

A better method to test the viscoelastic properties of muscles is to give a muscle a brief, short, quick stretch or quick release during different phases of contraction. In this way, a number of sophisticated phenomena have been detected.

HILL [31] was the first who observed the tension response to the stretch by instantaneous rising and decreasing followed by asymptotic recovery (see Fig. 10c). Afterwards, this author [32] discovered that this effect is obvious for both states, twitch and tetani in the whole muscle just like in a single fibre. This research evidence, as well as a rise of stiffness during the latent period of contraction, Hill described by so-called "two-component model" [33]. The instantaneous response in tension was explained by stretching of the undamped "series-elasticity" (A in Fig. 11), and the recovery by the contractile element (B in Fig. 11). Such an approach quite well described the hyperbolic force-velocity relationship. However, Hill's model is rather conceptual; it does not take into account the changes in internal structure of the sarcomere, or mechanochemical events occuring during muscle contraction. The two-component model could not also satisfactorily predict a complex behaviour of tension recovery when the response is recorded with considerably improved time resolution [2, 20, 30, 34]. Yet, Hill's approach suggests that the elements responsible for the muscle contraction is also responsible for their mechanical properties.
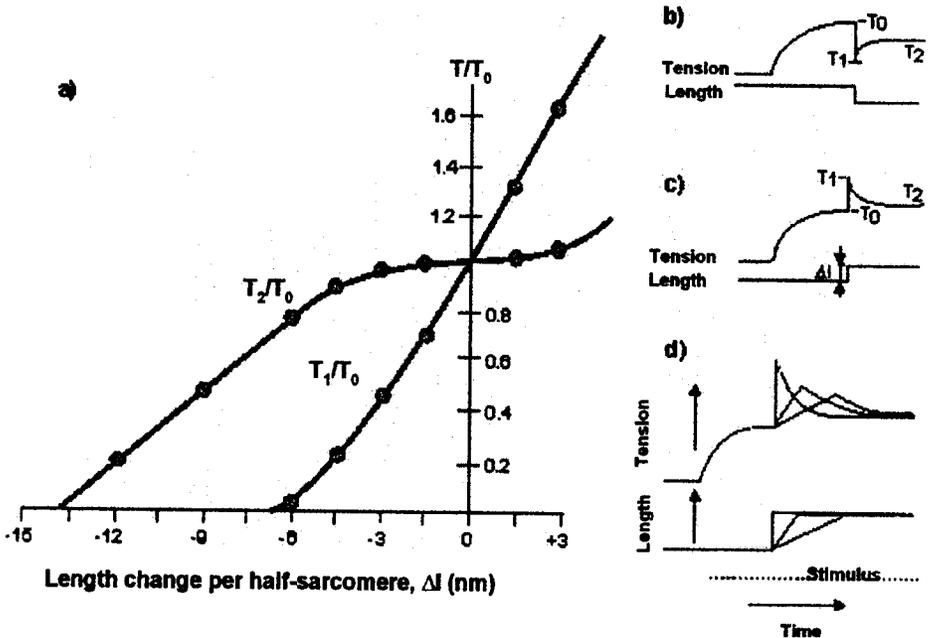
FIG. 10. The transient-state conditions.
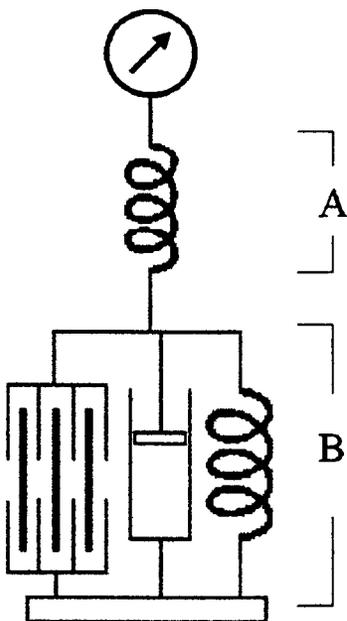
FIG. 11. The two-component model proposed by HILL [33].

HUXLEY and SIMMONS [2], who introduced the cross-bridge theory, have shown that the mechanical properties of muscles could be explained at the assumption that the cross-bridge consists of an elastic element in series with a second component having both the elastic and viscous properties. The viscoelasticity means that the cross-bridge should change its structure during contraction. At the assumption of the straight myosin tails, a possible structural change seems to be a shortening of both the subfragment-1 and subfragment-2. However, the computer simulation [15] shows that the real structure of the thick filament cannot be modelled from the straight tails. Moreover, the research evidence [35, 36] has showed that the actin and myosin filaments are compliant as the whole and not the cross-bridges alone.

More precise experiments [20, 34, 37, 38, 39] have revealed that the tension response is much more complex (Fig. 10). An instantaneous rise to a peak or a drop (from T_0 to T_1) during the quick stretch or quick release is followed by a complex tension decay or recovery to a plateau level (from T_1 to T_2). FORD et al. [39, 40] distinguished the four phases: Phase 1, the tension change during the step itself; Phase 2, a rapid, partial recovery to an intermediate tension level; Phase 3, a slowing of the recovery; and Phase 4, a gradual recovery towards the final tension level. Both tensions T_1 and T_2 depend on the size of the step Δl: if the imposed step is small, the tension T_2 recovers exactly to the tetanus tension T_0, and if the step is larger, the recovery tension will be proportional to the size

of the step. It has ben also revealed that the tension response depends on the speed of the length step (Fig. 10d): the smaller is the speed, the smaller and slower will be the response. The T_1 and T_2 intercepts lie at about 6 nm and 14 nm, respectively [39]. Moreover, it was shown that the values of T_1 and T_2 depend on the amount of overlap between the thick and thin filaments during the myofilaments sliding.

Interpretation of the foregoing results depends on the model used. In terms of the cross-bridge theory, the stiffness and tension are proportional to the number of the attached cross-bridges. So, it was concluded [1, 2, 27, 30, 34] that the early rapid component appears to occur without any appreciable amount either of detachment of the existing cross-bridges or formation of the new ones. The late stage of the tension recovering was interpreted by detachment of the cross-bridges followed by reattachment further along the thin filament. The process that re-stretches the elastic elements was described by a few steps of finite size. The stepwise movement was to be envisaged as taking place within the period during which a cross-bridge stays attached to a particular site on a thin filament. Moreover, in terms of the cross-bridge theory [40, 41], there are fewer attached cross-bridges at higher speeds of shortening and no increase in attached cross-bridges at the end of rapid velocity. PODOLSKY et al. [42, 43] have provided an alternative explanation. Such events as the response to abruptly changed length of an isometrically contracting muscle (quick-release), the end of the rapid recovery phase, as well as the tension excess during shortening relative to the isometric contraction, these authors interpreted by increasing of the number of the attached cross-bridges. There is also a theory of muscle contraction [44] according to which the sarcomere length changes not due to an action of the cross-bridges but due to both the myosin molecule melting and the elasticity of such structures as Z-discs, tendons, etc. Moreover, this theory asserts that the quick-release triggers synchronous behaviour along the whole fibre having some inhomogeneities within. Thereby, the imposed length change is absorbed mainly by the moving end of the fibre, less by its distal end, and the sarcomere-shortening step is delayed near the distal end of the fibre. The release from T_0 to T_1 defines the "series-elasticity" of the sarcomere, and the recovery from T_1 to T_2 results from the melting [44]. In consequence, for small stretch, the slightly shortened thick filament will be returned towards its initial length; for larger stretch, the thin filament will slide past the thick filament; if the stretch is substantial, connecting filament extension may be sufficient to contribute to the response. Recently the viscosity of muscles has been described [37, 38] by the friction associated with the movement not only of the cross-bridges but also of all constituents of the sarcomere.

The energy liberated during the muscle contraction also indicates that muscle is not a purely elastic body. FENN [45, 46] has found that the total energy (heat + work) released during shortening exceeds that released during isometric contraction. This effect, known in literature as the Fenn's effect, HILL [31] interpreted by the two-component model according to which the work is performed by the contractile element and the elastic element, being stretched, releases its elastic potential energy in the form of heat. The increase in the total amount of the released energy is proportional to both the total amount of shortening and the load. Additionally, if the muscle is prevented from shortening or is stretched during the contraction, the released energy decreases. The foregoing results clearly imply that contraction depends on chemical changes that in turn are regulated by the load and the extent of shortening permitted. In an elastic body, however, the total energy must be constant, i.e., there should be a simple exchange of heat for work, independently of the mechanical conditions.

5. A NEW INTERPRETATION OF THE MECHANICAL PROPERTIES OF MUSCLES

Some mechanical properties of muscles can be satisfactorily modelled from the point of view of their macro-structure [47, 48]. The review presented in the foregoing sections has shown, however, that the description of some muscle behaviour, even on the level of the muscle sarcomeres, may not be sufficient to understand them fully. The analysis performed by us by animation of the muscle contraction on the level of myofilaments suggests that the smaller unit responsible for continuous muscle work consists of seven thick and twelve thin filaments (see Fig. 12), i.e., of a group much smaller than the sarcomere. Moreover, an alternative model proposed by us [6] for the myosin molecules packing (see Fig. 2) gives rise to a new cross-bridge action [16] on the basis of which the muscle viscoelasticity becomes comprehensible.

In our model, after stimulation, the cross-bridges unwrap from the thick filament surface and move towards the surrounding thin filaments, each along a helical track (see Figs. 12, 13). The cross-bridge can unwrap up to a steady state, which depends on such circumstances like temperature, ionic strength, etc. Hitting into an encountered thin filament makes, however, impossible the steady state to be reached. Moreover, the interaction of one from two myosin heads with the appropriate actin monomer causes that the cross-bridge moves forwards together with the thin filament. Thereby the cross-bridge changes the track and moves along the filament axis until it reaches a minimum of the kinetic energy, i.e., a terminate steady state. The interaction between the actin and myosin can be strong or weak. The strong interaction can occur only if the specific binding-

sites on the myosin head and actin monomer meet each other (the rule of the stereospecificity). Due to the strong interaction, the thin filament may change its conformation: it may gradually elongate as the interaction gradually occurs [16]. This may give rise to additional sliding between the myofilaments what results in the cross-bridge pulling out from the terminate steady state. Thereby, because the myosin tail has the coiled-coil structure (see [49]), the stiffness within the subfragment-2 (yellow element in Fig. 13) will increase. Furthermore, the interaction induces the next steps in the ATP-hydrolysis cycle on each of the two heads [16], what may lead to a new electrostatic condition in the environment of the actin-myosin interface. All these coupled biomechanical and biochemical events should lead to detachment of the cross-bridge from the thin filament; consequently, the cross-bridge will come back onto the thick filament surface.
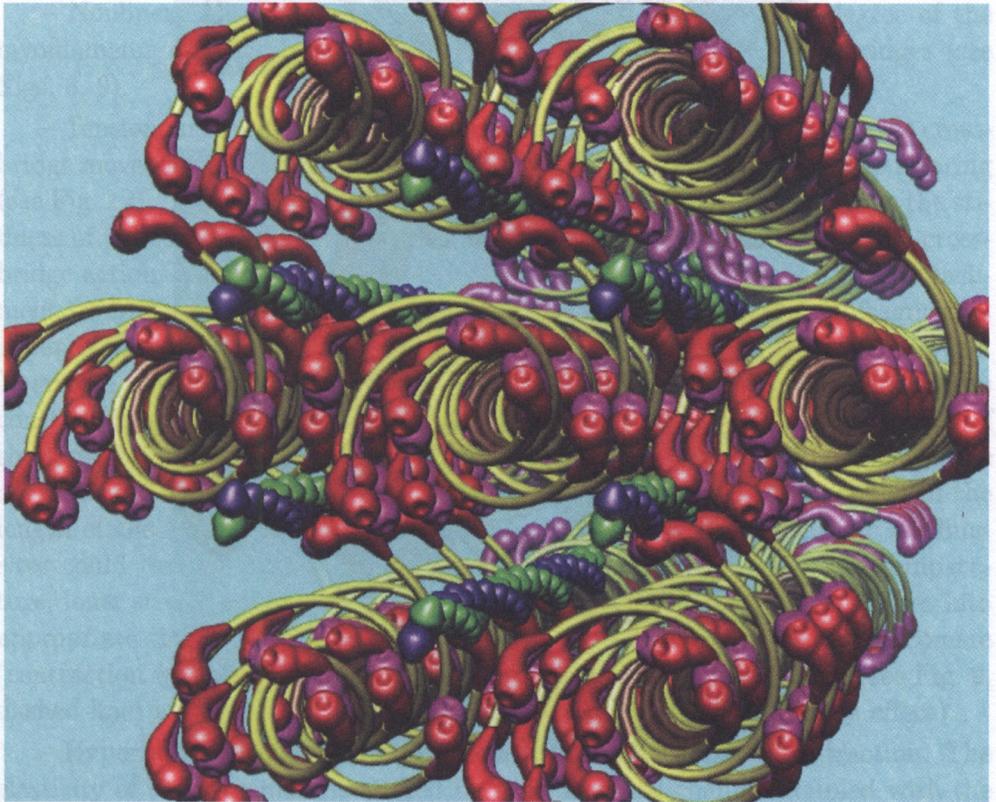
FIG. 12. The smaller unit responsible for muscle contraction. The group of seven thick filaments and twelve thin filaments is shown. Green and blue elements represent the actin monomers packed into the thin filament. The remaining elements are as in Figs. 1 and 2.
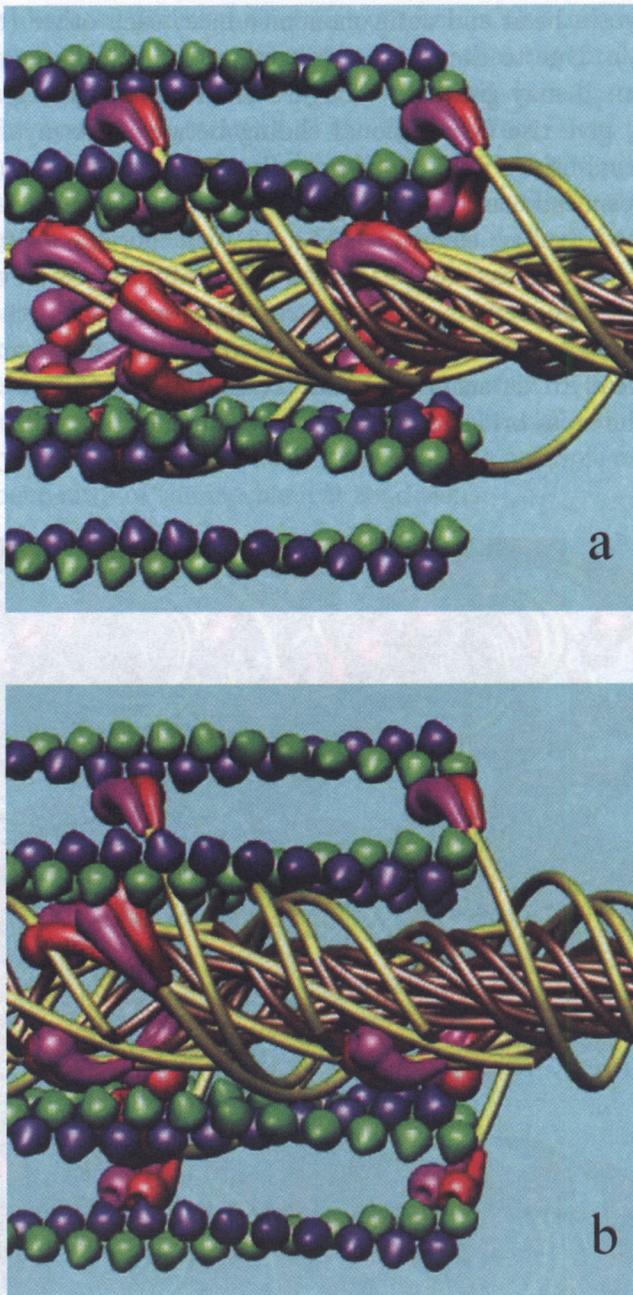
FIG. 13. The initial (a) and final (b) configurations of the cross-bridges during the phase of linking to the appropriate thin filaments. It is shown a part of the thick filament surrounded by six thin filaments. The elements are the same as in Fig. 12.

In our earlier paper [16] we have suggested that during contraction there are three groups of the cross-bridges working cyclically with the phase shift of T/3 (T is the period for action of each group). So, there are three principal phases for the each cross-bridge group: the phase of the cross-bridge linking with the surrounding thin filaments, the phase of detachment, and the phase of the cross-bridge arrangement on the thick filament surface. In Fig. 13a the moment is shown when the cross-bridges of one from the three groups hit the surrounding thin filaments, and Fig 13b depicts the configuration of the same cross-bridges in the moment of detachment. During this phase, the number of connected cross-bridges does not change although the myofilaments keep sliding.

The new manner of the cross-bridge action affords satisfactory interpretation of a number of the experimental data an understanding of which is difficult within the framework of the generally accepted concepts. It concerns first of all of the following research evidences:

– Nonlinear relationship between the consequently increasing degree of the myofilaments overlapping and the number of the interacting cross-bridges (see Figs. 8, 9).

– Tension and energy dependence on the state of the muscle. Due to the cross-bridge movement along the helical path the whole sarcomere behaves like a spring (see Fig. 12). The stiffness of the whole sarcomere increases mainly due to the stiffness of the subfragments-2, which in turn changes during the cycle of the cross-bridge action; it is maximal right before the moment of the cross-bridge detachment from the thin filament (see Fig. 13b). The stiffness of the subfragment-2 depends also on binding with the thin filament, i.e., whether it is strong or weak. Therefore, the stiffness in isometric conditions, when the sarcomere maintains its constant length, is smaller than during shortening due to a smaller number of the cross-bridges strongly bound to the surrounding thin filaments at the same degree of the myofilaments overlapping (see Fig. 9, dashed line). Moreover, if the length of the sarcomere is not fixed, the matching between the specific binding-sites could become optimal for prescribed circumstances such as the temperature, ionic strength, etc., only after some cyclic hitting of the cross-bridges into appropriate thin filaments. Thus, allowing for some shortening of the sarcomere (contraction without a servo-control) should lead to a tension increase (see Fig. 9, dashed line) as well as to increase of the release of the energy (Fenn's effect).

– Hyperbolic relation between tension (load) and velocity of contraction. The elasticity of the sarcomere is altered by the structural changes coupled with the cyclic action of the cross-bridges. Various stiffnesses of the cross-bridge at the beginning and end of the attached phase (see Fig. 13) give rise to various elasticities of the whole sarcomere; it will decrease as the degree of overlapping between the myofilaments increase. Next, due to the strong interaction with the successively

hitting cross-bridges, the two-stranded superhelix of the thin filament gradually elongates and rotates, what should lead to a decrease of the sarcomere elasticity. Moreover, the bending of the subfragment-1 or a change of the basic period of the thick filament, 14.3 nm, resulting from the interaction between the myosin molecules, may also result in altering of the stiffness as well as the elasticity. The foregoing structural changes ought to be susceptible to the external loads, temperature and ionic strength, and thus should manifest themselves as the viscosity. Therefore, increasing of the load during isometric contraction should lead to a more extensive destruction, in consequence to longer time of reconstruction of a suitable matching between the myofilaments. In the other words, the velocity of shortening (see Fig. 7) should be maximal at zero loads and become nonlinearly decrease with the load increasing. The same mechanism should lead to an excess of the released energy during shortening in comparison to the isometric conditions.

In terms of the new model the transient events (Fig. 10) also becomes be comprehensible. During isometric contraction, the tension in the sarcomere violently increases after stimulation, then runs slowly until it reaches a constant value (Fig. 6), i.e., the response becomes be gradual after an instantaneous reaction. However, if the tension were governed only by the cross-bridges, it would increase gradually right after the stimulation. The first fast phases seem reasonably to be explained by increasing the volume between the myofilaments due to the Ca^{2+} ions diffusing from the sarcotubular reticulum in the environment of the myofilaments (their concentration increases by about two orders of magnitude). The myofilaments are cross-linked even in the resting state. So, the violent increasing of the Ca^{2+} ion concentration in the gap between the myofilaments should increase the tension in the sarcomere. Afterwards, as the successive cross-bridges, activated by the Ca^{2+}, begin to act, the tension begins to gradually increase.

By reason of the physicochemical peculiarities of the cross-bridges, suggested above, the muscle sarcomere can be described as a biospring the elasticity and viscosity of which change in time with a specific duration. The response of such a spring depends of cause on the relation between the specific duration and the duration of an external disturbance. Therefore, at quick stretch (see Fig. 10c) or quick relase (see Fig. 10b) the muscle should behave like an usual spring, i.e., the tension should, respectively, increase or decrease. In case when the stretch is slower, the structural changes within the cross-bridge keep pace with. That should result in increasing of the viscosity, consequently in a smaller and slower tension increase (see Fig. 10d). Fixation of the length after the external disturbance makes impossible a suitable matching between the myofilaments; thus the number of the strongly bound cross-bridges cannot reach the optimal value only some one. The intermediate number occurs gradually. In consequence, the recovery of tension from T1 to T2 occurs also gradually.

6. Concluding remarks

Summarising, we want to emphasize a number of aspects that could be particularly helpful in the future numerical modelling of the mechanical properties of muscles, and especially in understanding the muscle contraction mechanism:

– In the *in vitro* experiment the mechanical features of muscles are usually examined on a macro-level that depicts however an averaged result from the events on a micro-level.

– The interaction between one myosin head and actin filament is indispensable for force generation but is not sufficient for muscle contraction.

– Mechanical properties of the contracting muscle are fairly complex and the molecular events responsible for them are not yet clearly known. However, it is now clear that purely mechanical approach is not sufficient; physicochemical events as conformational changes in molecules and myofilaments, or change of electrostatic field along the myofilaments, should also be taken into consideration.

– The unit responsible for the muscle contraction is responsible also for mechanical properties of the muscle. Until now it has usually been assumed that the sarcomere is such a unit. Our analysis, based on the animation of muscle contraction [50, 51], suggests that the relevant unit is much smaller; it consists of a group of seven thick filaments and twelve thin filaments.

– The interpretation of the experimental data always depends on the model of the molecules packing taken into consideration. The prevailing model is based on the assumption that the straightening myosin tails place themselves nearly parallel to the thick filament axis (see Fig. 1), and consequently the cross-bridges work in the oar-like manner, i.e., by a bending in three hinge domains of the myosin molecule. Such a model is however unable to explain a series of experimental data, for instance, the viscoelastic behaviour of muscles.

– The new model of the myosin molecules packing into the thick filament (see Figs. 2, 12, 13), being consistent with the experimental data concerning the geometrical parameters (discussed elsewhere), gives a possibility to describe the viscoelastic properties of the stimulated muscles in absolutely natural manner. It presents also a sound foundation for working out a mathematical theory of the force generation in muscles.

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