centrifuge) in a linear sucrose density gradient 20–60% w/w prepared in TNE. The gradient was fractionated in a LKB Uvicord apparatus, aliquots of each fraction were taken and acid-insoluble radioactivity was measured in a Packard-Tricarb liquid scintillation counter. Figure 1A shows that the virus bands at the density of 1.19 g/ml.

The gradient fractions with the density of 1.18 to 1.20 g/ml were collected, diluted in TNE to the density of 1.10 g/ml, and the virus was sedimented at 150,000 *g* for 1 h RNA was extracted by phenol saturated by TNE, precipitated by ethanol with 20 µg/ml of carrier yeast RNA and stored at −20 °C. For characterization of the viral RNA, the latter was resuspended in TNE and centrifuged in a linear sucrose density gradient 5–20% prepared in TNE (20,000 rpm for 18 h in a SW 27.1 rotor of the same centrifuge). Figure 1B shows that RNA of TBEV sediments at 45 S.

DNA from HEp2 cells non-infected and chronically infected with TBEV (Hep2-Sof system) was isolated as follows. The cells were suspended in TNE, disrupted in a Dounce homogenizer, the nuclei were sedimented at 1000 *g* for 5 min and thereafter washed twice by centrifugation (5000 *g*, 10 min) through 0.25 *M* sucrose prepared in TNE. DNA was extracted by phenol and 0.5% sodium dodecyl sulfate and precipitated by ethanol. Thereafter DNA was resuspended in TNE, passed several times through a tuberculin syringe and RNA admixture was hydrolyzed by 0.5 *M* NaOH at 37 °C for 16 h. After neutralization, DNA was precipitated by ethanol and stored at −20 °C.

RNA-DNA hybridization was performed in formamide with subsequent centrifugation of the products in caesium sulfate gradients[8]. DNA was taken in excess (0.3–1 mg) and was denaturated by heating at 90 °C for 2 min followed by cooling in ice-water. ^3H labeled viral RNA was mixed with DNA in 50% formamide with 0.4 *M* NaCl in the volume of 0.2–0.4 ml, the mixture was incubated at 37 °C for 16 h and thereafter centrifuged in caesium sulfate density gradients (1.36–1.72 g/ml) in a Ti50 rotor of a Spinco 3 centrifuge at 35,000 rpm for 60 h .The gradients were fractionated and acid-insoluble radioactivity was counted as above.

Three repeated experiments gave similar results, one of which is shown in Figure 2. It is seen that TBEV RNA bands at the density of 1.65 g/ml in caesium sulfate gradients (Figure 2A, D G). Hybridization of the virus RNA with DNA from non-infected HEp2 cells does not change essentially the distribution of the radioactive label in the gradient (Figure 2B, E, H). Hybridization of the virus RNA with DNA from chronically virus-infected HEp2-Sof cells essentially changes distribution of the radioactive label: a part of it bands at the density of 1.45 g/ml that is characteristic for DNA, labelled (in our case) with fragments of RNA, and another part of the label occupies a zone with the densities of 1.60 to 1.52 g/ml that is characteristic for RNA: DNA hybrids (Figure 2C, F, J).

The data presented allow to conclude that the genome of HEp2-Sof cells chronically infected with TBEV contains DNA sequences homologous to the virus RNA, while such sequences are absent in the genome of non-infected HEp2 cells. Incorporation of DNA-transcripts of the virus RNA into the cellular genome may be an additional mechanism for the chronic persistence of the virus in the cells. In our case transcription of the virus RNA into double-stranded DNA might be due to the presence of a latent Oncornavirus in HEp2 cells, as has been already mentioned. Whether it is a peculiar phenomenon inherent in the system studied, or a more general mechanism of some chronic viral infectious, this is the subject of our further study.

ВЫВОДЫ. Исследована система клеток (НЕр2-Соф), в которых более 13 лет поддерживается хроническая персистенция вируса клещевого энцефалита. В опытах молекулярной гибридизации РНК вируса клещевого энцефалита с ДНК из ядер клеток НЕр2-Соф установлено наличие в геноме этих клеток последовательностей ДНК, гомологичных вирусной РНК. Эти последовательности отсутствуют в незараженных клетках НЕр2.

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The Velocity-Dependence of Myosin Cross-Bridge Movement and Tension Development in Oscillatory Contractions of Insect Fibrillar Muscle

Movement of myosin cross-bridges, pulling upon attachment to the actin the thin filaments towards the centre of the sarcomere, has been generally implicated in the mechanism of active tension generation[1–3]. Evidence for such an axial movement of the cross-bridges was derived from intensity changes in the 145 Å meriodonal reflection in activated vertebrate skeletal[4] and insect flight muscle[5,6]. When the 145 Å reflection was monitored during sinusoidal length changes at a power-producing frequency of 4 Hz it was found that the intensity varied inversely in phase with the active tension[6]. Thus, under the conditions investigated the extent of the axial movement of the cross-bridges was directly proportional to the amount of tension developed.

To study the kinetics of cross-bridge movement in relation to tension development insect fibrillar muscle has been allowed to perform sinusoidal extension-and-release cycles at different frequencies and amplitudes. By recording the intensity changes in the 145 Å reflection some information has been obtained as to the number of cross-bridges changing their angle with the myosin filament axis[4–6] at any particular moment in response to

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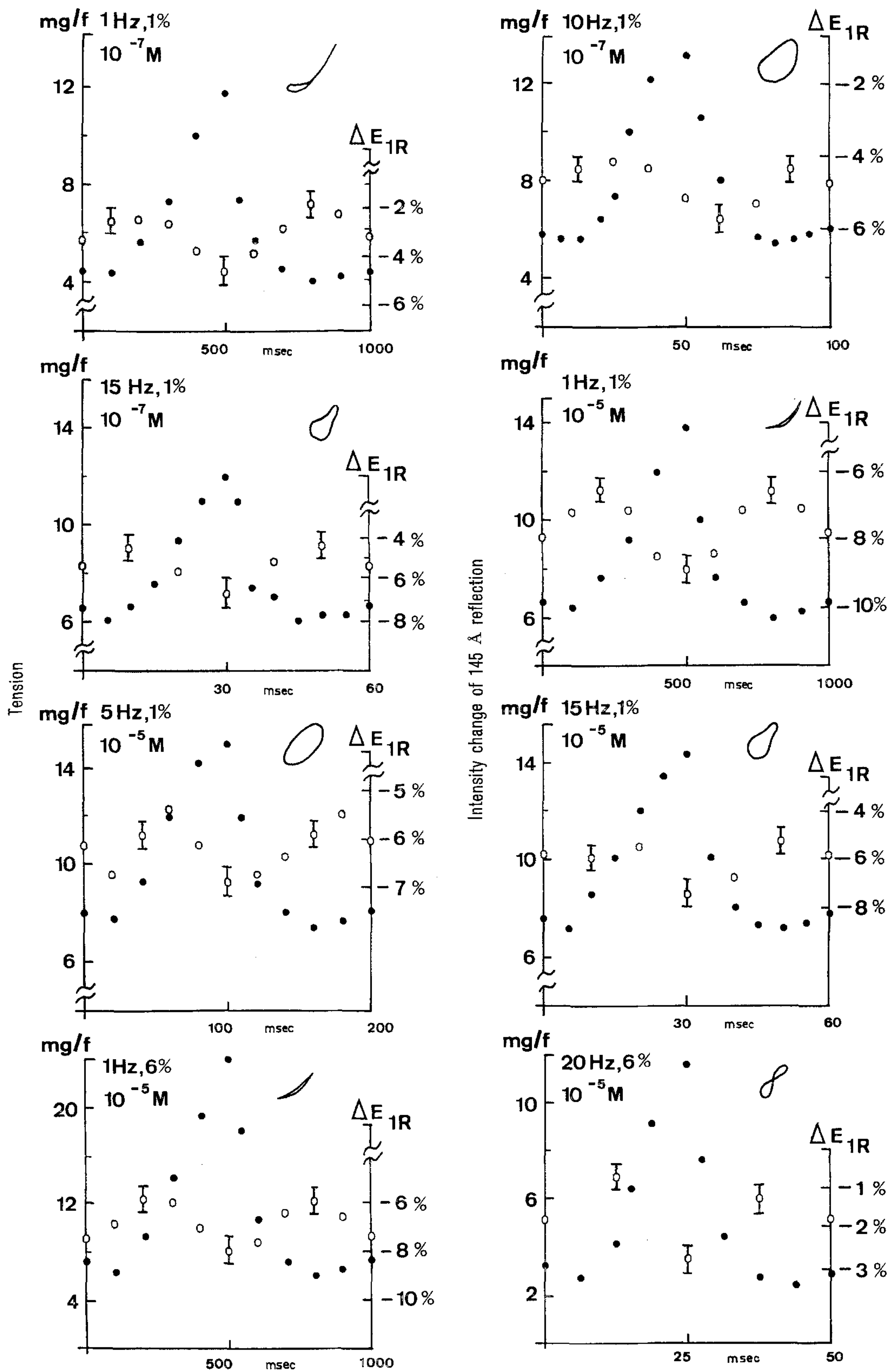

Variations in intensity of the 145 Å reflection and of tension with time during sinusoidal oscillation of water bug muscle. The intensity changes are indicated by the open circles, those of the tension (in mg/fibre) by the solid symbols. Error bars of the 145 Å changes are given. The oscillation frequencies and peak-peak amplitudes as well as the Ca^{2+} concentration are indicated in the Figure. For comparison the characteristic length-tension loops are included.

higher and lower velocities of length changes undergone by the muscle in presence of 10^{-7} and 10^{-5} M Ca^{2+}.

Material and methods. Bundles of 20 freshly glycerinated fibres from the dorsal longitudinal muscle of the giant water bug *Lethocerus colossicus* were glued at a free fibre length of 5 mm onto a pair of glass rods, one connected to a strain gauge tension transducer the other to a vibrator[7]. The fibre bundle was immersed initially in a relaxing solution: 15 mM ATP, 7 mM $MgCl_2$, 5 mM Na-azide, 5 mM EGTA, and 20 mM K-phosphate buffer, pH 6.8; the ionic strength was adjusted to 0.12 with KCl. The fibres were then extended by 1% and 4% above the length where they were no longer slack and transferred at this new length to the activating solution, the Ca^{2+} level of which was stabilized by a CaEGTA/EGTA buffer[7,8] at either 10^{-7} or 10^{-5} M.

For the X-ray measurements a Huxley-Holmes-type rotating anode X-ray tube and a camera similar to that of HUXLEY and BROWN[4] was used. The X-ray beam cross-section was collimated before the specimen to a spot of 150×180 μm. The specimen was oriented to give maximal absorption with the aid of a mirror positioned at 45°. The entire intensity of the innermost meridodonal spot, the 145 Å reflection (which could be obtained by placing a lead mask in the focal plane) was measured by sampling the direct beam (120×280 μm) with a digital counter[5,6]. The originally strong 145 Å intensity in the relaxing solution was recorded for each bundle as reference value, giving an average counting rate of 5740 counts per minute (cpm). The counts recorded every 10, 12.5, 20 or 100 msec of the oscillation cycle were accumulated for 20 min periods and the aggregate of counts compared with the active tension. The results were corrected for a background counting rate of 32–56 cpm. The intensity changes at 145 Å during sinusoidal oscillation in activating solution have been expressed relative to the intensity of the first equatorial reflection defined as $\Delta E_1\check{R}$. The first equatorial layer-line represents a reproducible high-intensity standard which remains essentially unchanged from one muscle to another, while in contrast the initial intensity of the 145 Å reflection in the relaxed muscle fibres can vary between 15–23% $E_1\check{R}$. By plotting only relative changes in terms of fractional intensities of the $E_1\check{R}$ the problem of a variation in the base line intensity is circumvented. In absolute terms the 145 Å intensity decreased between 4–40% under the conditions given in the Figure.

Results. In sinusoidal extension-release cycles of activated muscle information on the mechanical properties can be obtained from the shape of the length-tension loop and the amount of active tension developed. The velocity of the relative filament displacement between the actin and myosin filaments can be calculated directly, as it is the product of the rms-value of the peak oscillation amplitude times the angular frequency. In the experiments shown in the Figure the effective velocities of the imposed contraction-relaxation cycles have been varied between 0.11 mm/sec at 1 Hz and 1% peak-to-peak (p-p) amplitude, 0.665 mm/sec at 1 Hz, 6% p-p, 1.65 mm/sec at 15 Hz, 1% p-p, and 13.3 mm/sec at 20 Hz, 6% p-p. At the free fibre length of 5 mm the latter velocity corresponds to a shortening velocity of 2.6 muscle lengths/sec. As the cross-bridges, originally at right angles to the myosin filament axis, change their angle upon attaching to the actin[4–6], it is only to be expected from previous findings that whenever oscillatory tensions are produced the 145 Å intensity should decrease (see Figure).

At the lower velocities the changes in active tension and in the 145 Å intensity varied inversely in phase, as reported previously[5]. This effect was independent of the Ca^{2+} concentration, although the number of cross-bridges undergoing an axial movement is almost doubled on raising the Ca^{2+} concentration from 10^{-7} to 10^{-5} M (Figure). When the velocity was increased to 0.7–1.5 mm/sec the maximum of the intensity decrease was progressively lagging behind the tension maximum in the course of the oscillation cycle (see for example the 10 Hz data in the Figure). However, at higher velocities the 145 Å intensity and the tension change approximately in antiphase again, not considering certain asymmetries for half the oscillation cycle.

While at 10^{-7} M Ca^{2+} maximal work equal to 0.27 μcal fibre/min was produced at a frequency of 10 Hz, this was shifted to 15 Hz at 10^{-5} M Ca^{2+} with 0.69 μcal/fibre/min work performed. Thus, a maximal intensity change which at 10^{-7} M occurs at 15 Hz and at 10^{-5} M Ca^{2+} at 1 Hz does not coincide with maximal power output. Further, the amount of tension developed is by no means proportional to the changes in 145 Å intensity, if one for example compares the values for 1 Hz, 1% and 10^{-7} M Ca^{2+} and for 20 Hz, 6% and 10^{-5} M Ca^{2+} which give similar tension maxima.

At maximally activating Ca^{2+} levels of 10^{-5} M the number of cross-bridges changing their angle during the contraction and relaxation phase (as indicated by the maxima and minima of the intensity changes) decreases towards higher velocities. Before the muscle becomes passive again and no further intensity changes at 145 Å can be detected there is a critical velocity at about 2.5 muscle lengths/sec where the fibres are doing work for only half the oscillation period so that the length-tension loop has the shape of a figure-of-eight (Figure). If one calculates[5,6] the minimum fraction of cross-bridges which must move axially by about 100 Å to give the observed maximal intensity decreases the extreme values are 10% at 20 Hz, 6% p-p and 35% at 1 Hz, 1% p-p in presence of 10^{-5} M Ca^{2+}, being 18% at 1 Hz, 1% p-p and 10^{-7} M Ca^{2+}.

Discussion. The results, clearly provide a critical test for any chemomechanical model of muscular contractions, as the splitting of ATP must be closely linked to the cyclic cross-bridge movement[4]. Both the Ca^{2+} level and the velocity of muscle shortening and lengthening have been shown to affect the relation between tension development and the change in cross-bridge angle. At the low levels of 10^{-7} M Ca^{2+} the greatest extent of cross-bridge movement occurred at the higher velocities of relative filament displacement while at 10^{-5} M Ca^{2+} most cross-bridges changed their angle at the lowest imposed velocity.

At oscillation frequencies of 1–5 Hz the tension and intensity changes may be in phase because the cross-bridges are synchronized with the myosin remaining actually attached to the actin over the whole cycling period. At imposed oscillations of 10 Hz some of the myosin bridges may be forcibly stretched either dissipating their energy as heat or using it for the resynthesis of ATP[8] before they are able to generate an active force. Such cross-bridges would fail to contribute to the total tension produced whilst completing their operational cycle. At oscillation frequencies of 15–20 Hz there may be yet another synchronizing effect for the high velocities of the sinusoidal length changes may decrease sufficiently

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to allow cross-bridge attachment only when the muscle passes through its static mean length.

Zusammenfassung. Röntgenstrukturanalysen zeigen, dass die Beziehung zwischen sinusoidaler Kraftentwicklung und der Kinetik der Myosinbrückenzyklen von der Oszillationsgeschwindigkeit abhängt.

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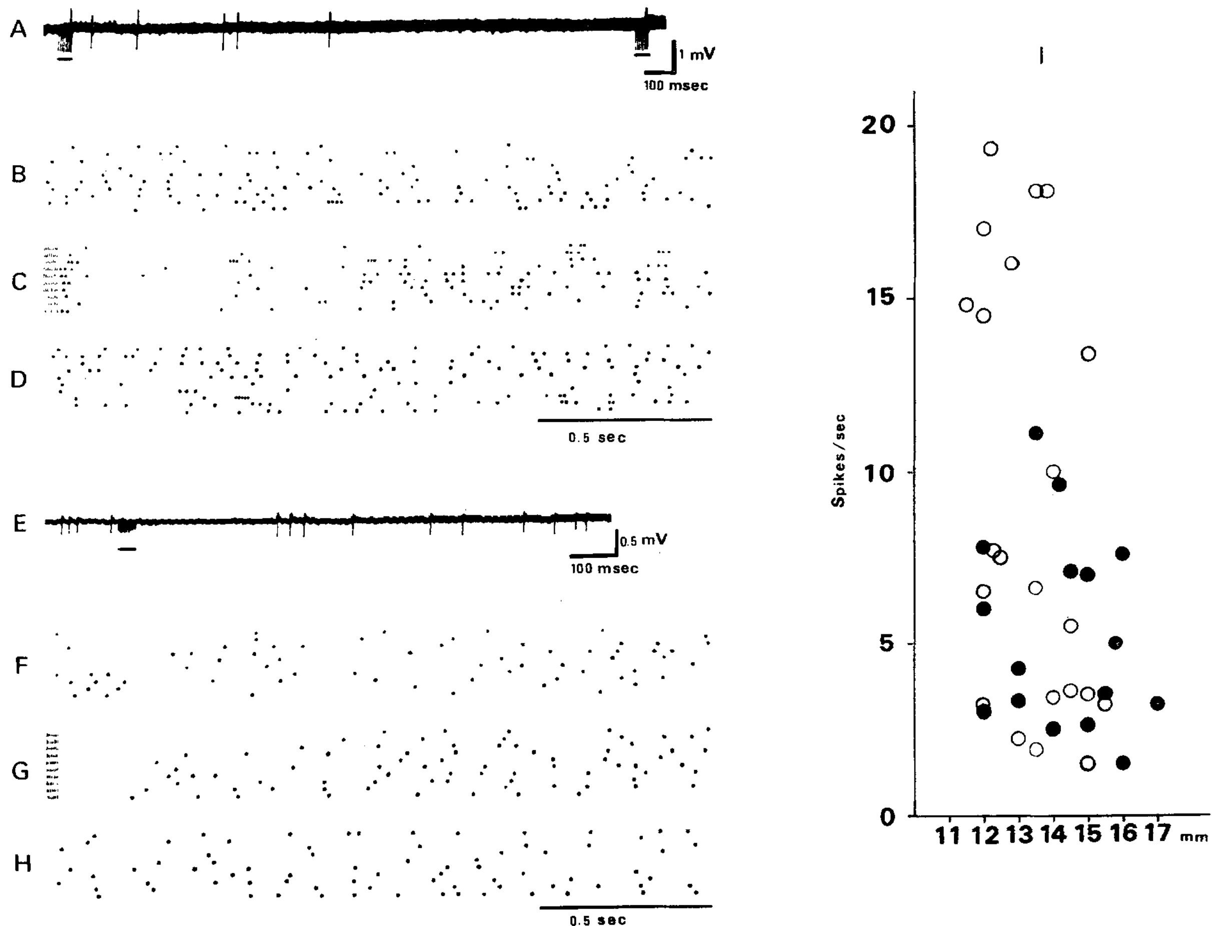
Excitation and Inhibition of Hypothalamic Neurons by Cerebellar Stimulation in Rabbits

A number of investigations have shown autonomic responses to be caused by stimulation of the cerebellum[1-3]. However, there is no agreement as to whether the hypothalamus is involved in the production of these responses[4-8]. The present experiment was undertaken to reveal influences of cerebellar stimulation on unitary activity in the hypothalamus.

Methods. 17 adult rabbits were used. They were anesthetized with i.v. injection of urethane (0.5 g/kg). After fixing the head of the rabbit in a stereotaxic apparatus, a bipolar electrode of stainless steel wire with a tip separation of 1.0 mm was inserted into the cerebellum. It was kept in place at a depth at which brief electrical stimulation at 100 Hz produced most prominent pupillar dilatations.

Electrical stimuli, consisting of 0.1 msec square waves repeated at 100, 200 or 300 Hz, were delivered once per 2 sec for a 30 to 50 msec period. ECG recording showed that this cerebellar stimulation caused no changes in heart rate. At the end of each experiment, an electrolytic



A) A sample record showing excitation. Cerebellar stimulations are marked by short horizontal bars. Single spikes were evoked toward the end of stimulus train. Positivity, downward. B), C) and D) Dot displays of spike discharges from another neuron. B) and D) control records taken before and after C), respectively. C) Early excitations followed by long-lasting suppressions due to cerebellar stimulation taken with 8 pulses at 200 Hz. E) A sample record showing inhibition. F), G) and H) Dot displays of spike discharges from another neuron. F) and H) Control records taken before and after H), respectively. H) Suppressions of spike discharges, immediately consequent upon cerebellar stimulation with a train of 8 pulses at 300 Hz. I) A plot of spontaneous firing rates of hypothalamic neurons as a function of the recording depths measured from the cortical surface. Open circles, excitation (number of units, 22). Filled circles, inhibition (16).