ASA activity (mU/ml) in the plasma of dogs in hemorrhagic shock ($n = 52$)

Period	Control	Hypotension		Post-reinfusion		
Sample No.	1	2	3	4	5	6
Mean	11.04	7.59	7.23	8.49	9.98	10.74
Median	9.58	6.24	6.24	7.29	8.33	8.74
20th percentile	7.20	5.04	4.99	5.12	6.08	6.58
80th percentile	13.49	9.33	8.91	9.96	12.58	14.62
P [a]		$P < 0.05$	$P < 0.01$			

[a] Probability of random occurrence of difference from the control value.

when blood from the reservoir was reinfused intravenously Following reinfusion the animals were observed for an additional 90 min. Blood samples (5 ml) were taken in the control period, in the 10th and 80th min of the hypotensive period and 30, 60 and 90 min after the end of reinfusion. ASA activity was determined in plasma by the method of BAUM, DODGSON and SPENCER[9] using *p*-nitrochatechol sulphate in acetate buffer, pH 5, as substrate and an incubation period of 180 min. Statistical analysis was done by the method of WILCOXON[10]. Percentiles[11] were used to estimate variation.

Results are summarized in the Table. It can be seen that ASA activity in plasma shows a decreasing tendency already at the beginning of the hypotensive period, and is significantly lower than control values by the end of the period. The low ASA activity begins to rise somewhat after reinfusion and reaches approximately control values at the end of the experiment.

We can offer the following two hypotheses for the interpretation of these results: 1. Release of ASA occurs from organs the perfusion of which is severely diminished during shock, and thus lesser amounts of enzyme can reach the general circulation. 2. During shock, inhibitors are released which decrease the activity of ASA in plasma. We have devised and commenced experiments in an attempt to study these possibilities.

Zusammenfassung. Bei 52 Hunden wurde in einem 90 Minuten dauernden Blutungs-Schock mit einem Blutdruck von 40 mm Hg eine 30%ige Verminderung der Aktivität der Arylsulfatase A im Plasma beobachtet.

G. HORPÁCSY, S. NAGY and T. BARANKAY

Institute of Experimental Surgery, University Medical School of Szeged, P.O. Box 464, Szeged (Hungary),
10 July 1973.

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Optical Diffraction Studies on Stimulated Single Fibres of Frog Muscle (*Hyla caerulea*)

When striated muscle is stimulated, the actin and myosin filaments slide together producing a contraction of several percent in each of the sarcomeres, the repeating structural units about 2 μm long which contain the filamentary mechanism. Because of the regularity of these sarcomeres the muscle acts upon an incident beam of light as a diffraction grating with a spacing equal to the length of 1 sarcomere. Observations of changes in the diffraction pattern thus enable changes in sarcomere length to be followed during muscular action[1-4].

By direct ciné-photography of the diffraction pattern during tetanus CLEWORTH and EDMAN[2,3] investigated the presence of the fluctuations in the sarcomere length which had been reported by LARSON et al.[4] and by GOLDSPINK et al.[5]. We have employed the same technique to determine the rate of filament sliding during an isometric contraction and to elucidate the nature of sarcomere length changes during a twitch.

Methods. Single fibres were dissected from the dorsal part of the semitendinosus muscle of a tree climbing frog (*Hyla caerulea*) and mounted horizontally (between a tension-transducer and a rigid arm) in frog Ringer's solution (NaCl, 115 mM; KCl, 2.5 mM; $CaCl_2$, 1.8 mM; Na_2HPO_4, 2.15 mM; NaH_2PO_4, 0.85 mM; pH 6.9) at 7 °C. A laser beam was directed upon the fibre at right angles to its axis and the zero and 1st order diffraction lines were displayed on a screen about 40 cm away. A moving-film camera focussed on the screen provided a continuous record of the spacing between the diffraction orders during activation. The fibre was stimulated transversely by a pair of platinum wire electrodes (0.007″ diameter) located immediately adjacent to the laser beam, so that the illuminated sarcomeres were the first to contract. A single 200 μ sec supramaximal pulse or a train of such pulses at an appropriate frequency was used to produce a twitch or a tetanus as required. The stimulating pulses were recorded on the film by including a synchronized light-emitting diode in the field of view (as seen in the Figure).

Results. Trace A in the Figure illustrates the typical sarcomere response in tetanus obtained from a region located midway along the length of a muscle. The resting sarcomere length was 2.6 μm and the temperature 7 °C.

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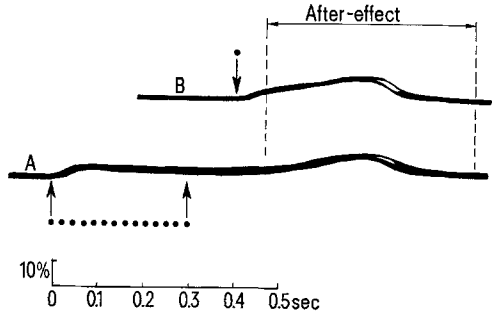
The initial contraction of 4.6% takes place at a rate of 150 (± 30) %/sec; with a sarcomere length of 2.6 μm this implies that the thin and thick filaments slide together with a relative velocity of 2.0 μ/sec. Although the photographic image of the 1st order line looks coarse and ill-defined to the eye, when it was scanned with an isodensitometer the changes in the position of the peak intensity could be determined, in favourable conditions, with 0.2% accuracy at minimum time intervals of 1 msec. Within this accuracy no detectable fluctuations of the first order line were present during the plateau of a fused isometric tetanus. There was no immediate change in sarcomere length when the stimulation ended, but 150 msec later the sarcomeres suddenly contracted by a further 5% (the 'after-effect'), and then entered the stage of slow relaxation to their initial lengths.

An instructive comparison may be made between this tetanic response and that of a twitch. To obtain the twitch response shown in the Figure, trace B, a single shock was applied to the muscle fibre immediately after trace A was obtained. The close similarity between the tetanus after-effect and the later stages of the twitch response is emphasized by the traces in the Figure which have been juxtaposed so that the peaks of the shortenings coincide. Similar comparisons were found from twitch and tetanus responses at other points along the length of the fibre.

Since the average sarcomere contraction over the whole length of a muscle fibre held isometrically must follow the changes in tension, there must be some sarcomeres which behave in a manner complementary to those sarcomeres responsible for the after-effects shown in the Figure. In general sarcomere polupations located near one or both ends of muscle have this property[3]. Thus, in the typical response of sarcomeres located near the tibial end of a muscle a large overshoot beyond the initial length was found to take place before the resting length is attained.



Sarcomere length changes of a stimulated muscle fibre recorded by displaying the zero and first order diffraction fringes on moving film. For reasons of space the zero order beam, which does not deflect on stimulation, is not shown on the record. Upward movement of the traces corresponds to a contraction of the sarcomeres. Dots below trace A and the dot above trace B are time markers produced by a light emitting diode synchronized with the stimulating pulses. Arrows indicate first and last pulses. Trace A) middle region of fibre tetanus. Trace B) middle region of fibre-twitch. The traces are aligned to emphasize the similarity of the after-effects.

Discussion. The main findings of the experiments described here are: 1. The initial rate of filament sliding in sarcomeres located near the electrodes is of the order of 2 μ/sec at 7 °C, which is close to the velocity of free isotonic contraction at this temperature[6]. This is to be expected in the experiments described because, as a result of the localized stimulation of the fibre, the sarcomeres being observed contract initially against virtually no load and achieve their maximum velocity of contraction before the external tension has significantly developed. 2. There are no detectable fluctuations of the mean sarcomere length at the time of a tetanus plateau, in agreement with the results of CLEWORTH and EDMAN[2,3]. It must be noted, however, that the 1 mm laser beam spans about 400 sarcomere lengths, so that this observation does not exclude the possibility that small asynchronous oscillations exist locally at the level of one or a few sarcomeres. 3. There is a long period with little or no local sarcomere length change following the end of stimulation. 4. There is a sudden further contraction of the middle sarcomeres (after-effect) following this latent period. As suggested by HUXLEY and SIMMONS[7] such behaviour can arise during the period of falling activity when some sarcomeres become unable to support the external tension and 'give', thus allowing the remaining sarcomeres to contract. 5. The form of the after-effect is essentially the same whether in twitch or in tetanus.

Zusammenfassung. Die Sarkomer-Bewegung während isometrischer Muskelkontraktion wurde mit Hilfe der Lichtdiffraktion untersucht. Die Anfangsgeschwindigkeit der relativen Bewegungen des Actins und Myosins betrug bei 7 °C etwa 2 μ/sec^{-1}. Nach Reiz-Ende verkürzen sich die Fasern noch etwas und zwar in der gleichen Art nach Tetanus wie nach Einzelzuckung.

J. BOREJDO, P. MASON and J. UNSWORTH[8]

School of Mathematics and Physics, Macquarie University, North Ryde (N.S.W. 2113, Australia), 13 August 1973.

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Effect of Manganese Ions on Human Platelet Aggregation in vitro

The platelet release reaction seems to involve mechanisms analogous to those involved in the contraction of muscle fibre[1] and has striking resemblances to the secretion process of chromaffin cells, mast cells and leukocytes[2]. As it has been proved that calcium ions are the sole triggering agent for the contraction and secretion mechanisms[3,4], it is worth studying the effects on platelet aggregation of agents interfering with the activity of calcium ions. This paper deals with the effects of manganese ions.

Materials and methods. Platelet aggregation was measured at 37 °C by the turbidimetric technique of BORN and CROSS[5] using an EEL Long Cell Aggregometer mod. 169. The 1.2 ml system consisted of 0.8 ml of citrated human platelet-rich plasma (PRP, about 3×10^8 plat/ml), 0.2 ml of adenosine-5-diphosphate trisodium salt (ADP) or noradrenaline bitartrate (NA) and 0.2 ml of manganese chloride or 0.2 ml of sodium chloride (control). The final concentrations of reagents, as salts, in the systems were as follows: 1.2 or 2.0 μ*M* ADP, 4.0 μ*M* NA, 20.0 m*M*