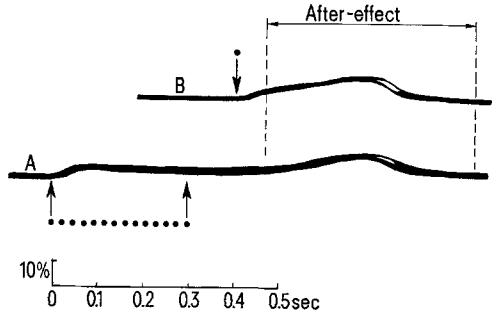
The initial contraction of 4.6% takes place at a rate of 150 (± 30) %/sec; with a sarcomere length of 2.6 μm this implies that the thin and thick filaments slide together with a relative velocity of 2.0 μ/sec. Although the photographic image of the 1st order line looks coarse and ill-defined to the eye, 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.

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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*

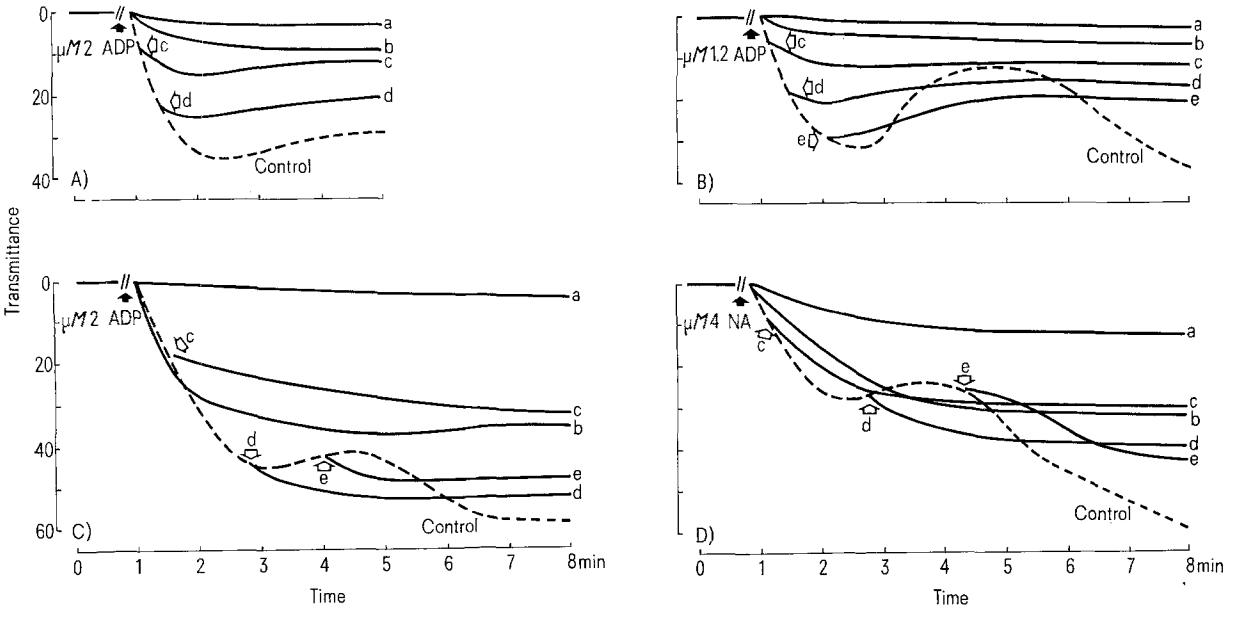

Fig. A–D. Inhibiting effects on human platelet aggregation in vitro induced by ADP and noradrenaline of manganese chloride m*M* 20 after 10 min incubation (a), when added together with the aggregating agent (b) and during aggregation at arrows (c), (d) and (e).

manganese chloride, 30.8 m*M* sodium chloride. The pH of the various systems measured at 37 °C using a Radiometer pH Meter mod. 25. ranged from 7.5 to 8.0. All trials were carried out at 37 °C within 2 h of preparing the PRP according to the technique already described[6].

Results and discussion. Manganese chloride added to 0.8 ml of PRP and 0.2 ml of sodium chloride was devoid of any effect. After incubation with PRP for 10 min manganese prevented the effect of the aggregating agents but it was more effective on ADP-induced aggregation (Figures A–D). When added together with ADP or later, the test substance blocked the aggregation in samples which showed both one-phase (Figure A) and two-phase aggregation (Figure B). The effect of manganese was slightly different when a higher concentration of ADP was added to the same sample of Figure B. In fact, although appearance of the second phase was always prevented, inhibition of the first phase was not complete when manganese was added together with ADP (Figure C) as the degree of inhibition depends upon the concentration of the aggregating agent. Results similar to those shown in Figure C were obtained using noradrenaline as aggregating agent (Figure D).

Manganese appears to be more effective on the second phase which is closely correlated with the platelet release reaction[7,8]. This secretion process is calcium-dependent, and the critical role of this ion fits the hypothesis that a contractile system (thrombostenin) is responsible not only for clot retraction[9] and for the platelet disc shape[10,11] but also for the process of aggregation and adhesiveness. The suggested parallelism between excitation-contraction coupling in muscle and stimulus-secretion coupling[2,4] seems to be accepted in that common ions appear to have similar effects on the two processes[2]. For instance, manganese ions, which are strong inhibitors of electrical and/or mechanical phenomena of muscle fibre in various experimental situations[3,12–15], also inhibit platelet aggregation. The mechanisms of action suggested for manganese ions in the former process could be applied to the latter. They occupy the sites of the membrane normally occupied by calcium ion and/or depress membrane conductance to calcium ions[14] or inhibit calcium permeability of the membrane[16].

Riassunto. Gli ioni manganese inibiscono l'aggregazione in vitro di piastrine umane indotta da ADP e da noradrenalina ed in particolare la seconda fase. Il meccanismo consiste probabilmente in una interferenza con l'attività degli ioni calcio.

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