## The Decremental Propagation of the Action Potential and Loss of Mechanical Response in Frog Sartorius Muscle in the Absence of Calcium

In a previous work[1], the effects of calcium lack was studied on frog Sartorius muscle in respect to resting and action potentials and the mechanical response of whole muscle and single surface fibres. It was found that resting potentials fell, in the absence of calcium, to levels at which the cell was inexcitable. It was also found that, even before cells became inexcitable action, potentials were diminished and a fall of the mechanical output occurred. The loss of the mechanical response of whole muscle in calcium lack is therefore a complex phenomenon, where inexcitability of individual fibres and reduction of the intensity of response of excitable fibres play a part. The present investigation is aimed at further elucidating the mechanism by which the mechanical output of individual fibres is diminished in calcium lack.

*Methods.* Sartorius muscles of German *R. temporaria* were used at 0–1°C. Mechanical and electrical recordings were made by the same methods as used previously[1]. The composition of the solutions was also the same as in the previous work.

*Results.* If the duration of the active state in calcium lack becomes less than the time required for the action potential to propagate along the fibre, a loss of the mechanical output should result. Conduction velocity measurements were made by taking the first detectable signal from an external probe electrode, which was moved along the surface of the muscle. In two muscles no change of conduction velocity was found from a value of 0.9 m/sec even after 2 h without calcium nor after 1 h after replacing calcium. The twitch/tetanus ratio did not fall more than 20% in the absence of calcium. These facts almost certainly preclude the possibility of a reduction in the intensity of the active state near the stimulating electrodes before the distal end of the fibres has been activated. The action potential takes 30 msec to pass along the fibre and the normal duration of the active state is about 100 msec at 0°C[2].

It is possible, on the other hand, to demonstrate that the contractile system becomes progressively less activated as the distance from the stimulating electrodes increases. Muscles were mounted on a multielectrode assembly with alternate cathodes and anodes spaced 3.6 mm apart (cathode nearest the pelvic end). The muscle was stimulated with 2 msec shocks alternately with the whole array or with a single pair of electrodes at either the pelvic or the tibial end. Great care was taken to choose just maximal stimulation voltages initially, both when using the whole array and the single electrode pair. The ratio between the voltages used for the whole array and the single electrode pairs was maintained constant during the experiments while adjusting the voltage on the whole array to keep the response just maximal for the prevailing conditions. It was found that the ratio of the twitch with the pelvic electrode pair to the twitch with the whole array fell steadily after removing calcium and in the later stages, the same also occurred for the stimulation with the tibial electrode pair. These experiments indicate that in calcium deficiency there is a progressive diminution of the activation of the contractile system along the fibre length from the point of stimulation.

The earlier communication[1] reported the modification of the action potential in calcium lack, with loss of overshoot and slower rise time, and recently KOKETSU and NODA[3] have also reported similar findings. The above finding that fibres fail to be fully activated at points remote from the stimulating electrode made it of interest to see whether the action potential also became modified as it travels along the fibre. Action potentials were therefore recorded alternately at both 3 mm and 25 mm from the point of stimulation using an intracellular microelectrode and choosing surface fibres at random at each position. The muscle was stretched until there was very little mechanical activity and stimulated at the pelvic end by 1-msec shocks applied to a pair of wire electrodes. No difference was found in the action potentials recorded at the two positions in the presence of calcium. Considering 35 records of fibres with resting potentials exceeding 57 mV taken between $^1/_2$ and $1^1/_2$ h after the removal of calcium, it was found that smaller action potentials existed in the distal region than in the proximal region (means 47 mV and 66 mV respectively) and that they more frequently failed at the greater distance from the point of stimulation. Furthermore, the lowest action potentials were associated with the lowest resting potentials in accordance with EDMAN and GRIEVE[1] and KOKETSU and NODA[3].

It thus seems probable that the decrease in the mechanical output from a single fibre, before it has become completely inexcitable in calcium lack, is due to insufficient activation of the contractile system by the affected action potential. The action potential, decreased in size even near to the stimulating electrode, becomes still weaker during its propagation along the fibre and so probably cannot activate the contractile system properly; in the extreme case the distal end of the fibre may not be activated at all. A contributory factor to the loss of mechanical output may be that there has been a failure of some more intimate link with the contractile system. Evidence for a calcium dependent link beyond the excitation of the cell membrane in skeletal muscle is given in a subsequent communication.

*Zusammenfassung.* Die Herabsetzung der Kontraktionsleistung einer Sartoriusmuskelfaser des Frosches, bevor sie wegen Calciummangel elektrisch unerregbar wird, kann weitgehend mit einer ungenügenden Aktivierung des kontraktilen Systems durch das Abklingen des Aktionspotentials erklärt werden.

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[2] J. M. RITCHIE and D. R. WILKIE, J. Physiol. *130*, 488 (1955).
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## A Calcium Dependent Link Beyond the Electrical Excitation of the Membrane in Muscular Contraction

It has been reported by FRANK[1,2], in experiments with frog's toe muscle, that the potassium-induced contracture fails at a stage where there has been no diminution of degree of depolarization caused by the potassium as measured by the petroleum gap method. If it were

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[2] G. B. FRANK, J. Physiol. *151*, 518 (1960).