

81.5% of arrested interphase cells are in G^2 . The conclusion that Sevin induces mitotic depression by arresting cells in G^2 without affecting DNA synthesis is, therefore, inescapable. The arrest of cells in G^2 further suggests that this pesticide interferes with the cell cycle at that stage.

- 1 The authors are grateful to Prof. K.S. Bilgrami, Head of the Postgraduate Department of Botany, Bhagalpur University, for providing facilities and to Prof. B.N. Mookerjee for his valued suggestions.
- 2 S.S. Epstein and M.S. Legator, *The mutagenicity of pesticides*. MIT Press, Cambridge 1971.
- 3 T. Mohandas and W.F. Grant, *Can. J. Genet. Cytol.* 14, 773 (1972).

- 4 S. Amer, *Cytologia* 30, 175 (1965).
- 5 S. Amer and O.R. Farah, *Cytologia* 33, 337 (1968).
- 6 S. Amer, M.A. Himmouda and O.R. Farah, *Flora* 160, 433 (1971).
- 7 W.F. Grant, *Symp. Biol. Hung.* 12, 43 (1972).
- 8 S. Singh and S.K.T. Nasar, *Proc. 3rd. All India Cell Biol. Conf.* 39 (1979).
- 9 W.B. Storey, L.S. Jordon and J.D. Mann, *Calif. Agric.* 22, 12 (1968).
- 10 K.D. Wu and W.F. Grant, *Can. J. Genet. Cytol.* 8, 481 (1966).
- 11 K.D. Wu and W.F. Grant, *Cytologia* 32, 31 (1967).
- 12 B.D. Singh, Y. Singh, J. Singh, R.B. Singh and M.R. Singh, *J. Cytol. Gent.* 6, 125 (1978).
- 13 A.H. Sparrow, R.C. Sparrow, K.H. Thomson and L.A. Schairer, *Radiat. Bot. (suppl.)*, 5, 101 (1965).
- 14 J.P. Miksche, *Can. J. Genet. Cytol.* 9, 717 (1967).

Muscle crossbridge action in excitation and relaxation

P. Mason and H. Hasan

School of Mathematics and Physics, Macquarie University, North Ryde, NSW 2113 (Australia), 21 November 1979

Summary. Pulse-propagation measurements on a muscle stimulated into tetanus show that the stiffness develops earlier and starts to relax later than the tension. It is concluded that the myosin heads which move towards the actin filaments during excitation become mechanically attached to them.

X-ray diffraction experiments have shown that when a muscle is stimulated the crossbridges swing out from the myosin filaments towards the actin filaments, returning to their original state when stimulation ceases². However, an unexpected feature was revealed by more recent experiments^{3,4} with time-resolution of the order of 10 msec, namely that the crossbridges move out more rapidly than the rate at which tension is developed and reach their final distribution well before the tension reaches its maximum value. Yagi et al.⁵ have also shown that at the end of a tetanic stimulation there is a marked discrepancy between the fall of tension and the return of the myosin heads. Such discrepancies cannot be explored on the basis of the X-ray data alone as these measure only the proportion of crossbridges which are near to the actin filaments without necessarily being attached to them⁶. Yagi et al. therefore proposed that mechanical experiments such as stiffness measurements might resolve the issue. We now report pulse-propagation measurements which show that the stiffness of a stimulated muscle also reaches a maximum well before the tension is fully developed, and thus support the view that the crossbridges which move transversely outwards actually become attached to the actin filaments.

Methods. We used a pulse-propagation technique to obtain a direct measurement of the propagation velocity of a mechanical pulse over the central 30 mm (approximately), of a muscle specimen. Experimental details of this technique have been given elsewhere⁷. The stiffness of the specimen is calculated from the measurements as the product of the density and the square of the propagation velocity. There is considerable dispersion of the square-wave input pulses as they travel along the muscle but the measurements are made solely on the leading edge of the pulse, which propagates with a velocity of the order of 100 msec. The uncertainty in the arrival time of the transmitted pulse is of the order of 10 μ sec. This is much smaller than measured relaxation times (reciprocal rate constants) for the initial recovery of stretched muscle fibers⁸ so that the stiffness measured is a purely elastic or 'instantaneous' stiffness.

In the present work fiber bundles approximately 40 mm long from the sartorius muscle of the toad, *Bufo marinus*, were pinned horizontally between a tendon and the pelvic bone, in Ringer's solution maintained at $5 \pm 1^\circ \text{C}$. The pulse transmitting and receiving crystals were brought into contact with the specimen over the central 30 mm of its length, so that the compliance of the tendon and mountings plays no direct part in the result. However, these 'external' compliances do permit the muscle to contract by several percent during stimulation, an important difference from the truly isometric experiments carried out with the spot-follower of Huxley and his co-workers⁹.

Results. In the figure, a, the solid-line curve shows the rise in tension as a specimen is stimulated into tetanus; the dashed curve shows the corresponding increase in stiffness above its level in the resting state. The curves are normalized to their values in the steady state of tetanus. When the muscle is soft the accuracy of the velocity measurements is too low to decide whether or not the stiffness and tension start to rise simultaneously. However, it is clear that the stiffness starts to increase proportionately more rapidly than the tension.

Furthermore (in contrast to an earlier, less accurate experimental result⁷) it reaches its final tetanic value well ahead of the development of maximum tension. This result parallels the X-ray diffraction observations and raises the problem of why the tension is still incompletely developed and takes approximately a further 200 ms to reach its tetanic level.

In relaxation, as the figure, b, shows, the stiffness decline is close to a mirror image of its development. The stiffness remains approximately constant for 150 ms until the tension has fallen by 10%. Optical measurements indicate considerable heterogeneity inside the muscle at this time, some sarcomeres expanding while others are contracting. At about 150 msec the stiffness starts to decrease rapidly indicating that a net detachment of crossbridges has begun. After about 250 msec, corresponding to the inflexion in the tension curve, the situation is dominated by the simultaneous expansion and contraction in different regions of

the muscle as indicated macroscopically by Huxley and Simmons' results with the spot-follower¹⁰ and microscopically by optical-diffraction measurements^{11,12}. The 'discrepancy' reported by Yagi et al.⁵ refers entirely to this period and may thus reflect internal readjustments in the muscle structure after this gross disruption.

Discussion. On the conventional sliding-filament model of muscle action, the attachment of a crossbridge to a thin filament produces simultaneous increases in stiffness and in tension. The full development of stiffness well before tension, as shown in the figure, a, is thus an apparent anomaly.

This simple picture, however, presumes isometric conditions which are only obtainable experimentally by using a feedback system such as the spot-follower. In the present experiments the muscle sarcomeres start to contract rapidly after the first stimulus (at about 0.2% per msec) and slow down to the isometric condition as the tetanus develops¹². Detailed consideration of crossbridge dynamics shows that the ratio of stiffness to force will decrease throughout the development period as the relative velocity of the sliding filaments decreases to zero.

A useful crossbridge model for such considerations is that of Harrington¹³, in which the force is generated by a helix-coil transition in a specific section of the α -helical S-2 link. When this transition takes place the coil exerts a tension

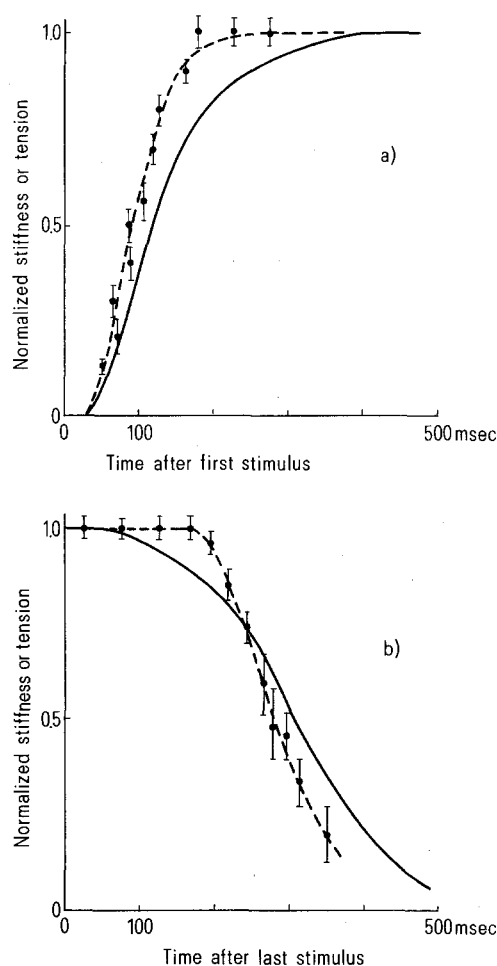
tending to bring its ends together. Mason¹⁴ has shown that for any intermediate distance, x , between the ends of the coil, the force is directly proportional to x , but the stiffness is a constant, independent of x . The ratio of stiffness to tension for a single Harrington crossbridge thus turns out to be simply proportional to x^{-1} . In the dynamic equilibrium which exists during a fully-developed tetanus the cycling crossbridges provide a constant average value of x , so that the stiffness:tension ratio is also constant.

In the developing phase of tetanus, however, the sarcomeres are shortening and each attached crossbridge is performing a finite stroke in displacing the actin filament relative to the myosin. At the onset of stimulation the contraction proceeds at constant velocity so that the time-average of x will be one half of its value at the instant of melting, i.e. one half of the length it occupied when in the α -helical configuration. Recent measurements on isolated S-2 fragments of myosin in solution have shown that between 15% and 25% of the molecule exists in the coiled form under physiological conditions¹⁵. The distance, x , between the ends of the coil at the instant of melting is thus approximately 20% of 65 nm, the length of S-2 in the fully α -helical form, i.e. 13 nm.

As the tension rises to the equilibrium tetanic level the rate of contraction decreases to zero¹⁶. The length of the crossbridge stroke also decreases towards zero and the average value of x therefore increases from about 6.5 nm to the full 13 nm as in the resting crossbridge. Over the period of tension development the value of x doubles so that the stiffness:tension ratio should fall to one half of its initial value; the results shown in the figure, a, are consistent with this.

The differing rates of development of stiffness and tension may thus be seen as a consequence of the relative sliding of the actin and myosin filaments until the muscle becomes truly isometric. With this model the stiffness gives a direct measure of the number of crossbridges which are attached; the tension is also proportional to this number, but its value is reduced while contraction is occurring.

The relatively early development of stiffness compared with tension parallels the relatively early outward movement of the crossbridges^{3,4}. These experiments thus support the view that the crossbridge heads which X-ray diffraction shows as moving out towards the actin filaments are actually attached to the filaments.



Stiffness (---) and tension (—) of stimulated toad sartorius fibers at 5°C. a) during development of tetanus; b) during relaxation after tetanus. The error bars on the stiffness points (\pm) indicate ± 2 SEM calculated from 9 experiments.

- 1 We gratefully acknowledge financial help from the Australian Research Grants Committee.
- 2 J.C. Haselgrove and H.E. Huxley, *J. mol. Biol.* 77, 549 (1973).
- 3 H.E. Huxley, 5th Int. Biophys. Cong., Copenhagen, S53 (1975); *Proc. Conf. on Fibrous Proteins*, Massey Univ. N.Z. Academic Press, in press (1980).
- 4 I. Matsubara and N. Yagi, *J. Physiol.* 278, 197 (1978).
- 5 N. Yagi, M.H. Ito, H. Nakajima, T. Izumi and I. Matsubara, *Science* 197, 685 (1977).
- 6 H.E. Huxley, *Cold Spring Harbor Symp. quant. Biol.* 37, 689 (1973).
- 7 H. Hasan and P. Mason, *Physics Med. Biol.* 23, 917 (1978).
- 8 A.F. Huxley and R.M. Simmons, *Nature* 233, 533 (1971).
- 9 A.M. Gordon, A.F. Huxley and F.J. Julian, *J. Physiol.* 184, 143 (1966).
- 10 A.F. Huxley and R.M. Simmons, *J. Physiol.* 210, 32 (1970).
- 11 D.R. Cleworth and K.A.P. Edman, *Science* 163, 196 (1969).
- 12 J. Borejdo and P. Mason, *J. mechanochem. cell Motil.* 3, 155 (1976).
- 13 W.F. Harrington, *Proc. natn Acad. Sci. USA* 68, 685 (1971).
- 14 P. Mason, *Biophys. Struct. Mech.* 4, 15 (1978).
- 15 K. Sutoh, K. Sutoh, T. Karr and W.F. Harrington, *J. mol. Biol.* 126, 1 (1978).
- 16 J.A. Barden and P. Mason, *Experientia* 35, 1584 (1979).