Striated Muscle Fibers: Inactivation of Contraction Induced by Shortening

Abstract. *The myofibrils in an isolated muscle fiber remain straight during the early part of a lightly loaded contraction initiated by membrane depolarization, but, as shortening continues, myofibrils in the core of the fiber become wavy, which suggests that their activation has been interrupted by shortening of the fiber. This may be a factor determining the length-tension relation at short muscle lengths.*

The force of isometric contraction and the speed of shortening decrease as the length of a vertebrate muscle fiber falls below slack length (*1*, *2*). One of several suggested explanations for the decline in contractility at short lengths is that the degree of activation is progressively less as a muscle shortens (*3*). We have photographed isolated frog muscle fibers while they were shortening under a light load and have obtained evidence that supports this suggestion.

The fibers were dissected from the semitendinosus muscle of *Rana temporaria* and mounted horizontally at slack length (that is, the length assumed by the unrestrained fiber) in a trough filled with Ringer solution at 20°C. The trough rested on the stage of an ordinary light microscope fitted with a Zeiss D* water-immersion objective (focal length 4.4 mm, numerical aperture 0.75) and a ×3 eyepiece. The illuminating cone was adjusted to numerical aperture 0.3. The resolving power with this arrangement was about 1 μm, so that what we see as an individual myofibril might be several. Stimulation was either transverse by a pair of bright platinum plate electrodes extending beyond the length of the fiber, or by a crossed-slot fluid electrode (*4*). With the latter arrangement, the fiber rested in a slot, 1 mm wide and 10 mm long, cut in a block of methacrylate polymer (Perspex). Another slot 1 mm wide, cut midway along and at right angles to the first, provided two symmetrically placed fluid cathodes. A glass cover slip rested on top of the block, separating the solution in the slots from that in contact with the objective. Negative current entered a small area of the fiber at the intersection of the slots and left over a large area to reach anodes that were distant from the fiber ends. Force was measured by an RCA 5734 transducer attached to one tendon of the fiber, and shortening was regulated by a lever-spring arrangement attached to the other tendon. When the fiber shortened against the spring the conditions were auxotonic, but the greatest load reached during shortening was only about 5 percent of the maximum isometric tetanic force. Cine micrographs (64 frames/sec) were made of an optical section through the center of the fiber about 1 mm from the stationary tendon in Figs. 1 and 3, and in the middle of the fiber in Fig. 2. The general techniques have been described in detail (*5*).

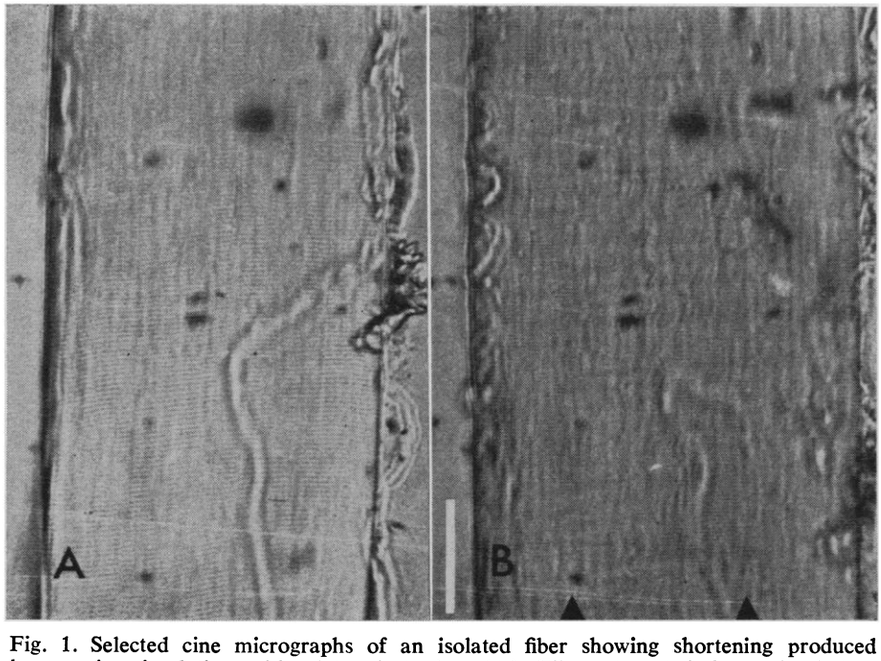

Fig. 1. Selected cine micrographs of an isolated fiber showing shortening produced by tetanic stimulation with plate electrodes. (A) Fiber at rest before stimulation; striation spacing 2.07 μm; (B) about 330 msec after the beginning of shortening induced by a train of 0.5-msec pulses at 50 hz. The arrows mark the approximate boundaries of the area in which wavy fibrils are visible. Temperature was 20°C. Calibration mark represents 50 μm. Exposure time 5 msec.

The fiber shortened from its slack length (Fig. 1A), and during the first part of contraction all the myofibrils remained straight. Then, about 70 msec after the start of stimulation, at an apparent (*6*) sarcomere length of 1.61 μm, the myofibrils in the middle of the optical section formed small waves about 15 μm long and 2 μm in amplitude. These were similar to the wavy fibrils previously described by Huxley and Gordon (*6*) who suggested, on the basis of the sliding filament theory (*3*), that the waves were produced when passive shortening is imposed on the fibrils by active shortening of another part of the cross-section of the fiber. This pattern (Fig. 1B) became slightly more pronounced; eventually it extended across about half the optical section and persisted throughout the period of stimulation (0.6 second). When stimulation ceased, the fiber resumed its original appearance, and when stimulation was repeated under isometric conditions at slack length, no waves appeared and the development and relaxation of force were the same as they had been before the shortening. So the wavy fibrils are not likely to be related to some irreversible change produced by extreme shortening, for example, delta state (*1*).

Similarly, the appearance of wavy fibrils (Fig. 1B) is not likely to be related to a possible failure of the action potential. The consequences of such a failure should also have been apparent in the force measurements, but the tetanic tension at slack length in these experiments ranged from 3.1 to 5.2 kg-wt/cm^2 during a maintained plateau. Furthermore, wavy fibrils can appear during shortening when the action potential is not the agent initiating contraction. When a fiber was made inexcitable by adding 10^{-7} g of tetrodotoxin per milliliter to the Ringer solution or by replacing the NaCl with choline chloride, a d-c stimulus of 100 to 500 msec duration delivered by the crossed-slot electrode produced a contraction like that shown in Fig. 2. The small part of the fiber where current depolarized the membrane shortened, while the much greater lengths of fiber on either side were stretched. A low

strength of stimulus produced a barely perceptible movement that we called a threshold response. The extent of shortening increased with each progressive increase in stimulus strength, and, at all strengths which produced measurable shortening, the entire cross-section of the fiber appeared to shorten actively. This is not surprising since a rise of only a few millivolts is needed to progress from threshold for contraction of the outer ring of myofibrils to threshold for contraction of the entire fiber (*7*). In this case we found, as we did during the tetanus, that all the myofibrils appeared to shorten actively only during the initial stages of contraction. When the fiber had shortened to a striation spacing of 1.6 μm, small waves formed in the center and extended over half to two-thirds of the optical section. When the fiber was stretched before stimulation, the waves still did not appear before shortening reached 1.6 μm. Thus the appearance of the waves was independent of the duration or total amount of shortening. When the plane of the optical section was set near the edge of a fiber, no waviness was seen. Thus there seemed to be an annulus of actively shortening myofibrils around an inactive core. Increasing the stimulus strength further did not diminish the area of waviness, an indication that the waves were not the result of insufficient depolarization of the fiber membrane.

Compressing a resting muscle fiber along its longitudinal axis produces wavy myofibrils that first appear at 1.9 μm (*8*). When a fiber is allowed to shorten from slack length under conditions in which not all of the myofibrils are activated by the electrical stimulus, the *unactivated* myofibrils first become wavy when the fiber shortens to a striation spacing of 1.9 μm (*5*).

In our experiments, wavy myofibrils did not appear before a fiber had shortened to a striation spacing of about 1.6 μm. We suggest, therefore, that all the myofibrils were activated in the early part of the contractions (Figs. 1 and 2), and that some myofibrils became *inactivated* by the process of shortening. Also, in contractures induced by potassium ion, during shortening wavy fibrils first appeared at a striation spacing of 1.66 μm, even when a fiber was 1.4 times slack length when shortening began (*9*). The interpretation of this result was different from the one suggested here. However, the fact that wavy fibrils did not appear before the fibers had shortened to about 80 percent of maximum on the curve relating the log of the K^+ concentration to shortening (*9*) is consistent with our view that extreme shortening induces inactivation that, in turn, leads to the appearance of wavy fibrils.

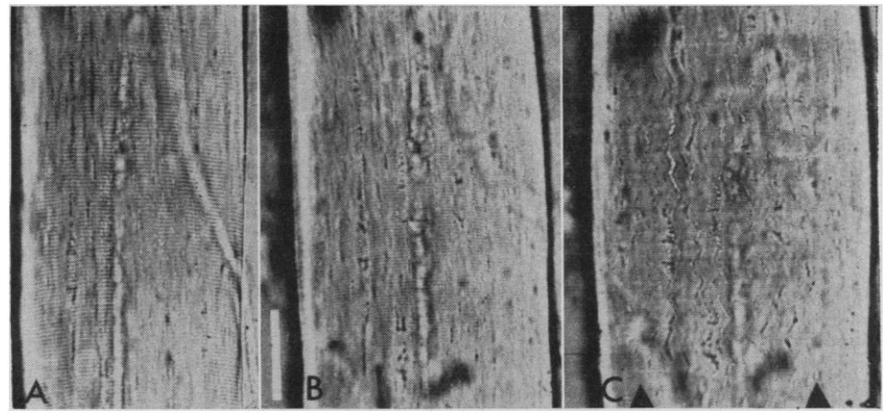

Fig. 2. Selected cine micrographs of an isolated fiber in Ringer solution containing 10^{-7} g of tetrodotoxin per milliliter, showing shortening produced by d-c stimulation with a crossed-slot electrode. (A) Fiber at rest before stimulation; striation spacing 2.15 μm; (B) during shortening, 50 msec after the beginning of a 500-msec pulse; striation spacing 1.71 μm; (C) during the plateau of shortening, 440 msec after the beginning of the pulse; striation spacing 1.36 μm. Other details as in Fig. 1.

There are several other phenomena which are possibly related to our observation. If a tetanized muscle is allowed to shorten considerably, application of a moderate load stretches it beyond the length where enough force should have been developed to hold the load (*10*). The degree of activation is apparently reduced, but this may have been induced by either the shortening or the stretch (*3*). Also, when a muscle is allowed to shorten during a twitch, its ability to lift a load has ended at a time when it could have developed considerable force had it contracted isometrically (*11*). Furthermore, if an isometrically contracting muscle is released to shorten at the peak of its force development during a twitch, heat production stops (*12*). These observations could be interpreted to mean that shortening somehow decreases activation in a contracting muscle.

The striation spacing at which the inactivation becomes apparent (1.6 μm) roughly coincides with the spacing at which the slope of the length-tension diagram suddenly changes (*2*). This is

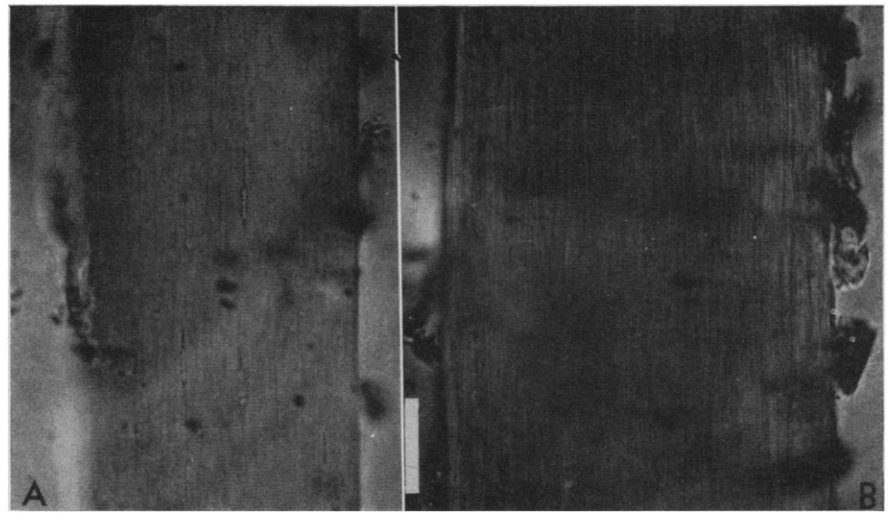

Fig. 3. Selected cine micrographs of an isolated fiber showing shortening produced by Ringer solution containing 1.5 m*M* caffeine. This is the same fiber as in Fig. 1. It has been rotated so that the optical plane is now along the minor axis of its elliptical cross section. (A) The fiber has shortened to a striation spacing of 1.54 μm with no waves apparent; (B) at a later stage, after a great deal of additional shortening, the myofibrils are still straight. Striations could not be resolved. This contraction was irreversible. The temperature was 5°C. Other details as in Fig. 1.

also the point at which the ends of the thick filaments should collide with the Z lines, thereby possibly producing a resistance to shortening, and if the thick filaments crumple or fold, the number of cross-bridges capable of generating force might be reduced (*2*). The disadvantage of using this to explain our observation is that one must postulate differences in length or compressibility of the filaments at different points along a radius of the fiber, for which, as far as we know, there is no evidence. Differences along a radius would also be required for an explanation related to an increase in the filament lattice spacing with shortening below slack length (*13*), or to an increase in the internal osmotic pressure (*2*), to the exhaustion of some energy supply, or to a facilitated removal of calcium.

Another possibility is that shortening somehow interrupts the inward spread of activation along the transverse tubular (T) system (*14*), or that some property of the T-system differs in different parts of a fiber. For example, the fact that the lumina of the tubules are two to three times larger near the fiber surface than they are deeper in the fiber may be significant (*15*). This last possibility is supported by the observation that the electrical event producing activation apparently spreads along the T-system near the fiber surface more readily than toward the center of the fiber under certain circumstances (*16*). We have also observed the pattern of shortening produced by immersing fibers in 1.5 m*M* caffeine, which is believed to penetrate the fiber membrane and to produce a contraction by releasing calcium from the sarcoplasmic reticulum (*17*). We found that a fiber in caffeine contracture shortens to well below 1.6 μm with no wavy fibrils in evidence (Fig. 3). This agrees with the possibility that the waviness induced by shortening to 1.6 μm in response to membrane depolarization is related to an interruption of the inward spread of the activating signal or perhaps to uncoupling between the T-system and the sarcoplasmic reticulum.

STUART R. TAYLOR*
REINHARDT RÜDEL

Department of Physiology,
University College London,
London, W.C.1, England

References and Notes

1. R. W. Ramsey and S. F. Street, *J. Cell. Comp. Physiol.* **15**, 11 (1940).
2. A. M. Gordon, A. F. Huxley, F. J. Julian, *J. Physiol.* (*London*) **184**, 170 (1966).
3. A. F. Huxley, *Progr. Biophys. Biophys. Chem.* **7**, 308 (1957), section V. 4.2.
4. R. E. Taylor, *J. Cell. Comp. Physiol.* **42**, 295 (1953); in *Physical Techniques in Biological Research* (Academic Press, New York, 1963), p. 237.
5. R. Rüdel and S. R. Taylor, *J. Physiol.* (*London*) **205**, 499 (1969).
6. The striation spacing is the same in the straight myofibrils near the surface and the wavy myofibrils near the center. It is not possible to determine the true sacromere length by measuring along the myofibrils with ordinary light microscopy [A. F. Huxley and A. M. Gordon, *Nature* **193**, 280 (1962)].
7. R. H. Adrian, L. L. Costantin, L. D. Peachey, *J. Physiol.* (*London*) **204**, 231 (1969).
8. H. Gonzalez-Serratos, *ibid.* **185**, 20 P (1966); electron micrographs of these fibers show that the waves originate in the myofibrils and not merely in some auxiliary component of the cell (L. M. Brown and H. Gonzalez-Serratos, unpublished observations).
9. H. Gonzalez-Serratos, *J. Physiol.* (*London*) **179**, 12 P (1965); thesis, University of London (1967), p. 57.
10. B. Katz, *J. Physiol.* (*London*) **96**, 58, figure 6 (1939).
11. B. R. Jewell and D. R. Wilkie, *ibid.* **152**, 30 (19€ → A. J. Brady, *Amer. Zool.* **7**, 603 (1967); *Physiol. Rev.* **48**, 570 (1968).
→ A. V. Hill, *Proc. Roy. Soc. Ser. B Biol. Sci.* **159**, 589 (1964).
13. P. W. Brandt, E. Lopez, J. P. Reuben, H. Grundfest, *J. Cell. Biol.* **33**, 255 (1967); G. F. Elliott, J. Lowy, B. M. Millman, *J. Mol. Biol.* **25**, 31 (1967).
14. A. F. Huxley and R. E. Taylor, *J. Physiol.* (*London*) **144**, 426 (1958).
→ L. D. Peachey, *J. Cell. Biol.* **25**, 209 (1965).
16. H. Sugi and R. Ochi, *J. Gen. Physiol.* **50**, 2167 (1967).
17. C. P. Bianchi, *J. Pharmacol. Exp. Ther.* **138**, 41 (1962); R. P. Gruener, *J. Physiol.* (*London*) **191**, 106 P (1967); A. Weber and R. Herz, *J. Gen. Physiol.* **52**, 750 (1968).
18. We thank A. F. Huxley for extending the facilities of his laboratory to us and for suggesting the use of the crossed-slot electrode, and we thank him and B. R. Jewell for reading and criticizing the manuscript. We thank G. Shaw for technical assistance. S.R.T. was a postdoctoral rehabilitation research fellow of HEW, and R.R. was supported by the Deutsche Forschungsgemeinschaft.
* Present address: Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn 11203.

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Harderian Gland: An Extraretinal Photoreceptor Influencing the Pineal Gland in Neonatal Rats?

Abstract. *The circadian rhythm of pineal serotonin and the influence of light on that rhythm have been confirmed. Removal of the Harderian gland abolishes the response to light in blinded animals, which suggests that this gland may act as the extraretinal transducer involved in the persistence of the pineal rhythm in blinded suckling rats.*

The serotonin content of the pineal gland undergoes a circadian rhythm with a maximum at about 1 p.m. and a minimum at 11 p.m. when lights are on from 5 a.m. to 7 p.m. (*1*). This rhythm persists in attenuated form in blinded animals and in rats kept in continuous darkness for up to 2 weeks (*2, 3*), but the nocturnal decline can be prevented if light is extended an additional 4 hours to 11 p.m. (*1, 3*). This effect of light on pineal serotonin occurs in blinded animals at 12 days of age (*4*), but not in blinded adults, which suggests the existence of a nonretinal photoreceptor influencing the pineal in suckling rats. Further, this receptor was localized in the head by the observation that hooding completely abolished the response to the additional lighting in 12-day-old, blinded rats (*4*). This photoreceptor has not yet been identified.

The Harderian gland, first described in 1694 in deer (*5*), is located behind and around the eye (Fig. 1). It is found in all vertebrates with the possible exception of higher primates; in some instances it is larger than the eye itself. Its function is unknown; speculation has ranged from a source of lubricant for the eye (*6*) to gonadal regulation (*6*) through merocrine secretion. Reddish porphyrins (primarily protoporphyrins) are present in this gland in the rat (*7*), and we have observed fluctuations in the porphyrin of the gland under different lighting conditions. We have used the following experimental design, essentially identical to that previously used (*4*) to examine a possible connection between the Harderian gland and the pineal serotonin content.

Newborn, male and female, Long-Evans rats were kept with their mothers, under a 6 a.m. to 6 p.m. lighting schedule, in plastic cages at a constant temperature at least 4 days prior to the experiment. All rats were blinded by complete bilateral enucleation or bilateral orbital enucleation combined with complete removal of the Harderian gland, carried out under ether anesthesia when the rats were 9 days old. Animals were blinded by pressing the sides of curved eye forceps on either side of the eye to force open the eyelids and push the eye forward. The forceps were closed about the base of the eye, and the eye and the attached optic nerve were pulled forward. By pushing the forceps somewhat deeper into the optic cavity, the eye and the Harderian glands could be removed simultaneously. After removal of the