Reports

Fatigue in Phasic and Tonic Fibers of Frog Muscle

Abstract. Single isolated phasic muscle fibers and small bundles of tonic fibers were directly stimulated in one-per-second twitch series by massive electrode shocks. During the stimulation period the isometric tension developed by the phasic fiber continuously decreased, first rapidly and then slowly. The tonic fibers behaved similarly, but showed much less fatigue than the phasic ones. In general, recovery of the fibers after cessation of stimulation also occurred in two phases.

It is known that striated muscle, when stimulated directly at frequent intervals, will fatigue (*1*). It has also been shown that single muscle fibers under direct stimulation will show a gradual decline of tension similar to fatigue of whole muscle (*2*). However, there is some controversy regarding this latter finding; Ramsey (*3*) states that gradual falling off of tension with continual stimulation of a single isolated fiber is due to injury, and that uninjured fibers will not show this gradual decline of tension with continual stimulation. Consequently, our work was undertaken to determine precisely the mechanical changes which take place in single phasic muscle fibers stimulated at frequent intervals, and also to compare the changes with those of similarly treated tonic muscle fibers. Since the underlying cause of fatigue is still unknown, these and future experiments will attempt to shed some light on this phenomenon.

Both single phasic fibers and small bundles (10 fibers or less) of tonic fibers were isolated from the semitendinosus muscle of *Rana pipiens*. The fibers were mounted horizontally by their tendons in a Lucite dish containing 60 ml of phosphate-buffered Ringer's solution with 0.2 percent *d*-tubocurarine. During an experiment oxygen was bubbled through this solution, and this served not only to oxygenate the tissue, but also to stir the medium constantly. All experiments were performed at 10°C. The developed isometric tensions were measured with a special low-drift myograph consisting of a combination of two RCA 5734 transducer tubes (*4*), and were recorded photographically from an oscilloscope. The fibers were mounted between two parallel platinum-plate electrodes so that the entire length of each fiber could be stimulated instantaneously. For these experiments, the fibers were maintained at rest length and stimulated once per second with slightly super-threshold square-wave shocks of 0.3 msec duration.

In all cases reported here the isolated fibers after 60 min of equilibration following dissection could be stretched and could develop tetanic tensions reversibly, and were thus considered uninjured. The phasic fibers had a diameter of 72 to 93 μ and developed isometric tensions of 0.9 to 1.2 kg/cm^2 (thus about 50 mg per fiber) before fatigue. The tonic fibers, being much thinner (about 25 μ in diameter), could not be individually isolated, and so we used small bundles which developed tensions of 50 to 125 mg. For this report, no attempt was made to determine the intrinsic tension output of the individual tonic fiber.

It was found for single phasic fibers that as twitches were evoked at the rate of one per second the developed tension continuously decreased in two phases. As shown for a typical experiment in Fig. 1, there was first a relatively rapid drop of 60 percent within 4 min, followed by a much slower decay which continued until there was no response. No staircase variations were observed, and the initial tension of the fiber remained constant throughout the stimulation. The recovery of the twitch output after the stimulus was removed occurred in two phases, a rapid restoration of 30 percent in 15 sec, followed by a slow recovery which approached the initial amplitude within 60 min.

The rise time of the twitch for phasic fibers, measured from the onset of tension to peak, increased during the fatigue process. For the experiment represented in Fig. 1, the rise time gradually doubled during the first 8 min of stimulation, going from about 55 to 110 msec, and then remained fairly constant. However, the relaxation time of the fibers, measured from peak to one-half of the developed tension, showed a very rapid and large increase in duration. As shown in Fig. 1, the relaxation time was doubled after 3 min of stimulation and continued to rise to four times the initial value after 9 min. As with the amplitude changes, the recovery of the relaxation time occurred in two phases, a rapid one followed by a slow recovery to the initial time. The recovery of the rise time did not show any rapid phase, but slowly approached the initial time.

The tonic muscle fibers presented a rather different behavior. During stimulation at the rate of one per second, the decrease in the twitch amplitude, though developing in two somewhat distinct phases, was at all times very much smaller than that of the phasic fibers.

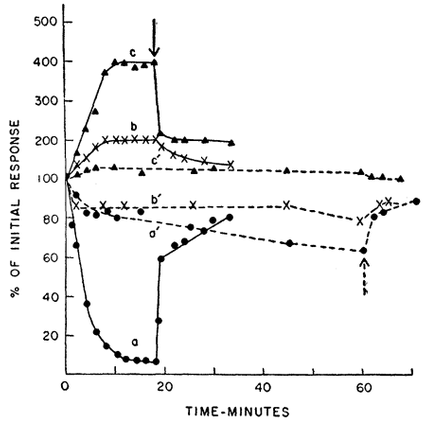

Fig. 1. Typical fatigue and recovery effects associated with a one-per-second twitch activity series of a single phasic fiber of 93-μ diameter (solid lines) and of a bundle of tonic fibers (dashed lines). Curves *a* and *a'* indicate changes of twitch amplitude; *b* and *b'*, rise time of twitch; and *c* and *c'*, relaxation time. The percentage changes are plotted relative to the response of the preparation just prior to the start of stimulation. The arrows mark the termination of activity and the beginning of recovery. (In cases where the fatigue of phasic fibers was followed in time beyond that shown here, the twitch gradually declined to zero.)

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

In a typical experiment as given in Fig. 1, after 4 min the twitch amplitude had dropped about 15 percent, and after 60 min it had declined only about 35 percent. After cessation of stimulation, the response increased 15 percent in 2 min and approached the initial amplitude at a slower rate. Both the rise and relaxation time remained fairly constant during the stimulation period, except for a change during the first few minutes; for the case shown in Fig. 1, the rise time decreased 15 percent during the first 2 min of stimulation, while the relaxation time increased 25 percent during the first 6 min of stimulation.

After each experiment, a potassium contracture (with 0.1*M* KCl) was recorded to check whether the fibers were phasic or tonic. Phasic fibers gave the usual contractures which relaxed after a minute or so, whereas the contracture of tonic fibers declined slowly over a period of many minutes.

Our finding that tonic fibers fatigue much more slowly than phasic ones confirms on the single muscle-cell level the previously made conclusion for such units derived from studies of the relative fatigability of corresponding whole muscles (*5*). Further research is required to determine whether there is any relation between this difference in fatigability and other fundamental properties distinguishing phasic and tonic fibers (*6*). The decrease in twitch output of a phasic muscle fiber occurs along a smooth curve until it finally fails completely to respond. Thus these fatigue changes and the corresponding ones we have observed in the tonic fibers are quite unlike the sudden total obliteration of response which Ramsey (*3*) describes, and which we also have seen, in a single fiber undergoing a tetanus of rather high frequency. Considering that the fatigue and recovery processes of the fibers occur, in general, in two steps, it would seem that fatigue may be attributed to two separate factors. The first may be due to changes in the excitation mechanism or in the excitation-contraction coupling, the second to changes at the level of the contractile mechanism. In elaboration of the results reported here, our future studies will deal with the effects of activity on the membrane process and with the role of individual fiber behavior in the development of fatigue of the whole muscle (*7*).

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References and Notes

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7. This research was supported by a grant from the Muscular Dystrophy Associations of America, Inc.

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Isolation of Abscisin, an Abscission Accelerating Substance

Abstract. A crystalline substance, designated *abscisin*, which accelerates abscission of excised debladed petioles at 10^{-2} microgram per abscission zone, has been isolated from cotton burs. The yield was approximately 1 milligram from 10 kilograms of dry plant material.

The presence in plants of a substance (or substances) which accelerates abscission has been reported by several investigators. Osborne (*1*) found that the diffusates from senescent petioles of several plants accelerated abscission. Biggs and Leopold (*2*) discovered a factor from senescent leaves and fruits which accelerated abscission of debladed petioles. Herrero and Hall (*3*) found that extracts of pulvinoids from abscising leaves of cotton accelerate leaf abscission. Carns (*4*) and Carns *et al.* (*5*) reported that extracts of young cotton fruit walls accelerated abscission of debladed leaves and of young fruits. These findings, together with other evidence, led Addicott (*6*) to suggest that the substance(s) represents a new type of plant hormone, one that accelerates abscission. This report announces the isolation and preliminary characterization of such a substance from cotton burs (dried, mature fruit walls after dehiscence and removal of seed and fiber).

The bioassay used to evaluate abscission-accelerating activity during the investigation was a modification of the explant test employed by Addicott *et al.* (*7*). Explants were cut from cotton seedlings when they were 18 to 20 days old. They consisted of 5-mm stumps of the cotyledonary petioles, 5 mm of the stem, and 10 mm of the hypocotyl. Test substances were applied in 1-percent agar as 0.005-ml droplets. Explants were held upright in stainless steel holders in petri dishes containing a layer of about 5 mm of 1.5-percent agar. Abscission was determined by the application of a uniform load of 10 g to the petiole stumps.

Approximately 134,000 ground cotton burs (364 kg) were extracted for 6 hr in batches with a total of 1725 liters of Skelly-solve B (petroleum ether, boiling point 60° to 75°C). The extract was concentrated to 9 liters in a falling-film concentrator. A 3-liter aliquot of this concentrate was extracted twice with 4.5 liters of a water-methanol (1:4) solvent, and the top lipid-rich layer was discarded. The methanol extract was taken to dryness and then exhaustively extracted with 5-percent sodium bicarbonate and filtered off, and the filtrate evaporated to dryness under reduced pressure. Subsequent extraction of the dried residue with anhydrous acetone yielded 10 g of an acetone-soluble solid which accelerated abscission in the explant bioassay at 5 μg per abscission zone.

A sample of 5.7 g of the active solid was subjected to partition chromatography on a silicic acid column (6 × 25 cm) that had been treated with sodium bicarbonate; water was used as the stationary phase (7 g of water per 10 g of adsorbent), and chloroform followed by *n*-butyl alcohol was used as the mobile phase (*8*). The butanol

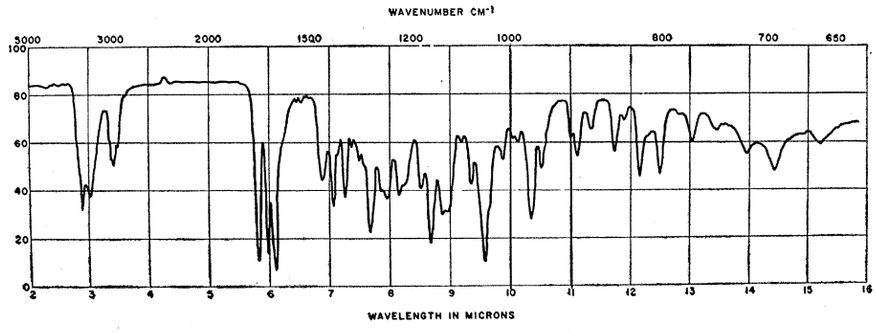

Fig. 1. Infrared spectrum of abscisin.