

Structural Changes in Single Muscle Fibers after Stimulation at a Low Frequency

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ABSTRACT Direct stimulation of single muscle fibers from *Xenopus laevis* at a frequency of 1 Hz results in a decline of the peak isometric twitch tension after about 200 twitches. Fibers were chemically fixed in glutaraldehyde after a varying number of twitches and at several fatigue levels, and the ultrastructural appearance was compared with that of resting fibers treated by identical fixation methods. No gross structural abnormalities were observed but subtle changes occurred. The mitochondria of stimulated fibers contain granules of normal size and number. The inner crista width is constant but the matrix width is increased on stimulation. These changes would not compromise ATP production. The myofibrils are normal except for a slight swelling in the myosin lattice. The transverse system (T system) and sarcoplasmic reticulum are intact. The minor diameter of the transverse tubule (T tubule) is increased slightly in stimulated fibers. The gap between the T-TC membranes stays constant at about 110 Å, but tiny connecting pillars are seen to cross this gap more frequently in stimulated fibers ($21 \pm 5\%$ triads) than in resting fibers ($8 \pm 6\%$). In stimulated fibers there is a marked increase in the electron dense content of the terminal cisternae (TC). Inasmuch as the observed structural changes correlate with the number of twitches but not with the fatigue level, it is concluded that TC density and T-TC pillar formation are related to the normal mechanisms of excitation-contraction coupling.

INTRODUCTION

Repetitive stimulation of muscle fibers causes the isometric contractile force of the twitch to decline. The muscle is then said to be fatigued. It has been proposed that muscle fatigue may be a result of some failure in the coupling between excitation and contraction possibly due to the swelling of the transverse tubules (T tubules) (Somlyo et al., 1978) or in the process of Ca^{++} release from the sarcoplasmic reticulum (Eberstein and Sandow, 1963). Evidence also indicates that fatigue may be related to a decrease in myoplasmic calcium due to mitochondrial uptake (Gonzalez-Serratos et al., 1974) and to depletion of ATP which is the energy source of calcium release and force generation (Vergara and Rapoport, 1974; Vergara et al., 1977).

The possibility that fatigue results from a failure of the contractile proteins has been ruled out because the muscle is capable of producing normal twitches when caffeine in subcontracture concentration is added (Grabowski et al., 1972).

Although it was found that fatigue is accompanied by a 20-mV shift of threshold to a more positive potential, the overshoot of the surface membrane action potential remains unchanged (Mashima et al., 1962).

It seemed likely that electron microscopy studies would help locate the structures in which fatigue occurs. We chose to look for changes after several minutes of low frequency (1 Hz) stimulation to single *Xenopus* muscle fibers. Higher frequency could have been used, but metabolic factors might then be more central (Vergara et al., 1977). A protocol was designed to examine stimulated fibers before the fatigue process had begun and thus to separate structural changes caused by stimulation alone from those of stimulation and fatigue.

A brief report of this work was presented by Gilai and Eisenberg (1977).

METHODS

Dissection and Mounting Procedures

Single fibers were isolated from M. flexor brevis digiti V of *Xenopus laevis* in Ringer solution. The tendons were cleared of dead fiber remnants and trimmed to ~ 1 mm in length. Only fibers which twitched in response to a propagated action potential were used.

A muscle bath with a Sylgard bottom (Dow Corning Corp., Midland, Mich.) contained 5 ml of unstirred Ringer solution. One end of the fiber was clamped firmly by its tendon, the other tendon was tied to a short glass hook attached to a strain gauge mounted vertically and held by the movable arm of a micromanipulator. After mounting, the fiber was stretched to 140% of slack length.

Stimulation

Massive stimulation along the entire length of muscle is necessary for synchronous activation. Rectangular pulses, 0.05 ms in duration, were produced by an isolated stimulator unit (Digitimer, type 2533, Devices Sales Ltd., Welwyn Garden City, Hertfordshire, England) and fed to a power amplifier stage (model 6842A, Hewlett-Packard Co., Palo Alto, Calif.). Stimulating current was applied through two platinum plates flanking the fiber along its full length, with an intensity of about twice threshold (Goodall, 1960; Mulieri, 1972). Repetitive stimulation was at a frequency of 1 Hz. All experiments were performed at room temperature (22–25°C) without temperature regulation in the bath.

Tension Measurement

A piezo-resistive strain gauge assembly (type 8206-Pixie, Endevco, Becton, Dickinson & Co., San Juan Capistrano, Calif.) was used in a bridge circuit whose output was proportional to force in the force range generated by a single fiber. This transducer is subject to slow base-line drift, probably due to small, local temperature changes. The problem of base-line drift was overcome by mounting the transducer in a heat sink and also by using an automatic DC-balance circuit developed by Nakajima et al. (1976). The bridge circuit output was fed into an amplifier (Teledyne Philbrick, Dedham, Mass., model 1027) with a gain of 23, then into the automatic DC-balancing circuit. The twitch was displayed on the oscilloscope and chart recorder simultaneously through a low pass filter of ~ 40- μ s time constant.

The peak isometric twitch tension varies from one twitch to the next during a train of stimuli at 1 Hz (Fig. 1). The initial peak isometric twitch tension (P_0), the maximal peak

twitch tension at the maximal level in staircase (P_m) and the peak twitch tension just before fixation (P_f), were measured and expressed in units of newtons. The numbers of stimuli needed to achieve P_m is designated as t_m (because the frequency is 1 Hz, this is also the time interval in seconds). For simplicity we tabulate $(P_m - P_0)/P_m$ as a tension ratio to indicate the potentiation of "staircase," and P_f/P_m as a tension ratio to indicate the degree of fatigue. (Note that P_f was somewhat arbitrarily determined). The rate in decline of the peak isometric tension is not linear and also varies from one fiber to another. This rate of fatigue was assessed by noting the time and number of stimuli taken for the tension to fall from P_m to half of P_m . This interval is called the fatigue half-time.

Fixation

The Ringer solution in the bath was removed and rapidly replaced with the fixative. Stimulation continued during the solution exchange. After the fixative reached the fiber,

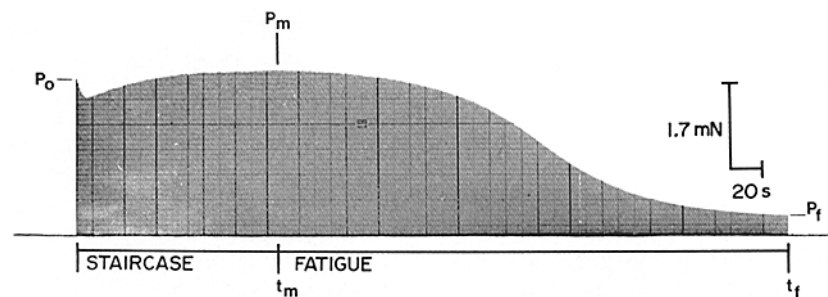


FIGURE 1. Fatigue demonstrated in a single muscle fiber from *Xenopus laevis* during a continuous train of stimuli at 1 Hz. P_0 , the initial peak isometric twitch tension; P_m , the maximal peak isometric twitch tension; P_f , the peak isometric twitch tension before fixation with glutaraldehyde. t_m , the number of twitches to reach P_m ; t_f , the total number of twitches.

we seldom observed a second twitch. This would indicate that the processes involved in excitation-contraction coupling are inactivated by glutaraldehyde in <1 s. In other experiments in our laboratory we have fixed single fibers maintaining a potassium contracture. In these cases it takes up to 5 s for the maintained tension to fall to zero. Thus, the fixative takes longer to penetrate inwards and inactivate the contractile proteins.

The structure of the fibers was examined by fixing the fiber in one of two stages: (a) the early stage where 200 or fewer twitches were produced; or (b) the fatigued stage where 300 or more twitches were produced. Fibers in the early stage were usually still in the state of maximal twitch potentiation, whereas in the fatigued stage the tension was considerably reduced.

The initial fixative was 5% glutaraldehyde, 2 mM CaCl_2 in 0.1 M sodium cacodylate buffer, pH 7.2, at room temperature. After 1 h the fibers were placed overnight at 4°C in 10% sucrose, 2 mM CaCl_2 , and 0.1 M sodium cacodylate, pH 7.2. In most cases the fibers were then cut into 1–2 mm lengths and processed on a watch glass viewed under a dissecting microscope. In some experiments collective processing was used to ensure identical treatment of the resting and stimulated single fibers. For identification the resting fibers were cut into short segments and the stimulated fibers into longer segments, (or vice versa) and all were then carried through remaining solutions together.

All segments were postfixated in 1% OsO₄ in the same buffer at room temperature for 1 h, stained in 1% uranyl acetate for 1 h, dehydrated, and embedded in Epon (Shell Chemical Co., New York). To produce longitudinal sections of single fibers, a careful embedding procedure was followed. Thus, double-sided Scotch tape (3M Co., St. Paul, Minn.) was placed on a sheet of glass and a drop of Epon placed on it. The short pieces of fibers were placed in the drop and after 2 h at 60°C a capsule was filled with Epon and inverted over the fiber until polymerization was complete. A short segment from a resting muscle and a long segment from the stimulated muscle could be placed side by side and hardened into a single block face. Thus, both fibers had been exposed to the identical heavy metal staining throughout processing, and therefore, any differences in density must be due to inherent fiber differences and not to preparative artifact.

Thin sections (gray to light silver) were cut on a LKB-Huxley microtome (LKB Instruments Inc., Rockville, Md.), stained with uranyl and lead citrate and viewed with a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N.J.). Micrographs of central and surface areas in several locations along the length of each fiber were taken. The print magnifications were between $\times 20$ and $\times 100,000$. Some grids were also examined with the Zeiss 10A electron microscope (Carl Zeiss, Inc., New York) fitted with a goniometer stage so that tilt series could be taken.

In a few fibers after the longitudinal block sections were made, the block was embedded again and cross sections were taken. These were used to determine myosin spacing at a known sarcomere length so that the myosin lattice volume (K_v) could be determined from the equation of April et al. (1971, p. 75).

T System and Sarcoplasmic Reticulum

To minimize bias, measurements were made from the micrographs without knowledge of the experimental protocol in each case. The sarcomere length was measured. The minor diameter of the T tubule was measured between the two cytoplasmic membrane leaflets because this parameter has a constant value in all T-tubule profiles of longitudinally sectioned muscle. The major axis was not measured because its value varies as a sine function of the angle between the section plane and the T-tubule axis. However, the frequency distribution of the measured major axis could be used to calculate the true major axis (Posakony et al., 1977). The total number of T-tubule profiles and the number of T tubules with abnormalities were counted. Any gross shape distortion, folding, or breakage in the membrane was classified as an abnormality. The total number of profiles of terminal cisternae (TC) were counted and most cisternae in the resting muscle had a uniform distribution of granular material within them. Any cisternae which had a dense accumulation of material close to the TC membrane which faces the T tubule was counted as a "dense TC."

The width of the gap was measured between the cytoplasmic surfaces of the T tubule and the TC membranes (T-TC gap). The measurement was made in the narrowest place which is usually in the region of the "feet" (Franzini-Armstrong, 1975, 1977). In each fiber, careful measurement of 100–200 T-TC gaps were made with a $\times 7$ magnifier containing a micrometer ruled in 0.1-mm divisions. The electron micrograph magnification was at least $\times 40,000$. In the process of making these measurements, a finer structure of the feet was observed in some triads. The feet did not always have the fuzzy density that is usually reported, but were sometimes well organized as tiny cylindrical pillars. The dimensions of the pillars and frequency of occurrence were recorded from all micrographs by an observer who did not know whether the fibers were stimulated or resting.

Mitochondria

Mitochondria are quite rare in frog muscle comprising only 1% of the cell volume (Mobley and Eisenberg, 1975). Certain micrographs were taken selectively to include mitochondria inasmuch as random selection of a muscle fiber area printed at $\times 50,000$ magnification rarely includes mitochondrial profiles. We wished to measure the changes in the matrix and inner crista spaces. Stereological analysis was not performed because this fractional determination requires that the fiber volume remain constant. Fatigued fibers are known to swell (Gonzalez-Serratos et al., 1974). Furthermore, in that single fibers are being studied and the mitochondria are rare, the condition of statistically random sampling required for stereology cannot be applied. We therefore used a linear index of matrix width and inner crista width (Fig. 2). A line was drawn on the mitochondria perpendicular to the cristae (Fig. 6). A calibrated magnifying lens was used to measure at least 10 widths from each mitochondria, and the mean value was recorded for further analysis.

Within each mitochondrial profile, a quadratic test grid was used to count points falling of the mitochondria (P_{mit}) and points falling of the mitochondrial granules (P_{gr}). The volume of granules per unit volume of mitochondria is given by the ratio of P_{gr}/P_{mit} .

The number of granule profiles (N_{gr}) was counted and the average profile cross-

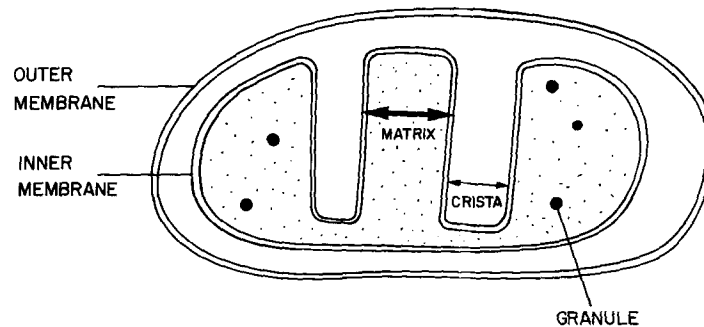


FIGURE 2. Diagram of a mitochondrion to define the cristal widths measured.

sectional area of one granule, A_{gr} , was calculated from $A_{gr} = g^2 P_{gr}/N_{gr}$, where g was the test grid space of $0.1 \mu\text{m}$. Because the granules are small spherical objects, the mean profile diameter d_{gr} can be calculated from the area of one granule $A_{gr} = \pi (d_{gr}/2)^2$, and d_{gr} can be used to calculate the true diameter of the granule (D_{gr}) by use of a stereological formula $D_{gr} = 4d_{gr}/\pi$ (Eq. 6.1 of Weibel, 1973).

The numerical density (N_v) of the number of granules (N_{gr}) per unit volume of mitochondria (V_{mit}) can be corrected for section thickness effects (Eq. 6.25 in Weibel, 1973), and then $N_v = N_{gr}/g^2 P_{mit} (D_{gr} + K)$, where K is the section thickness, assumed to be $0.1 \mu\text{m}$. The section thickness was estimated by the interference colors seen when the section is floating. The thickness is estimated to be between 0.07 and $0.1 \mu\text{m}$ in all sections. Systematic bias between resting and experimental muscle will not be introduced because the sectioning was always done in the same way. However, the absolute values could be in error for both muscles.

RESULTS

The time-course and degree of fatigability varied from one fiber to another. Therefore, the fatigue characteristics were determined on single fibers, and the

structural changes were studied on a fiber with a known history. In all we completed the analysis on six resting fibers, four fibers in the early stage, and seven fibers in the fatigued stage. The results of the fatigue characteristics will be reported first. The electron microscopic changes will be described for the membrane systems and then for the mitochondria.

Fatigue Characteristics

The peak isometric tension produced by a train of stimuli at one per second was measured. Tension and the time-course of fatigue varied considerably from fiber to fiber (Table I); a typical record is shown in Fig. 1. Not all fibers show the

TABLE I
FATIGUE CHARACTERISTICS* OF SINGLE MUSCLE FIBERS
STIMULATED AT 1 Hz

	Total number of twitches, t_f	Number of twitches to reach maximal tension, t_m	Staircase poten- tiation, $\frac{P_m - P_0}{P_m}$	Fatigue index, $\frac{P_f}{P_m}$
	n			%
Early stage (under 200 twitches)	120	—	0	100
	120	—	14	100
	180	—	32	100
	200	79	33	42
	\bar{x}		20	85
	SD		16	29
Fatigued (over 300 twitches)	320	73	14	14
	322	160	5	67
	329	110	13	47
	360	0	0	33
	482	175	0	56
	579	195	27	73
	1,054	95	33	23
	\bar{x}		115	13
SD		68	3	22

* See Fig. 1 and text for definitions.

negative staircase in the first few twitches or the staircase potentiation of the twitch. The maximum peak isometric twitch tension, P_m , usually occurs after 1–2 min with the mean value of t_m being 115 s. The degree of staircase potentiation varies between 0 and 33%. The rate in decline of tension also varied from one fiber to another over a large range (80–300 s for the fatigue half-time).

Electron Microscopy

Chemical fixation of the membrane surface is probably achieved in <1 s and the entire fiber is fixed in <5 s. In that all fibers were processed in the same way, the relative difference in structures must depend on stimulation and not on fixation effects.

The overall appearance of the ultrastructure of stimulated muscle fibers was

not grossly different from resting muscle. No swelling between myofibrils or of the membrane-bounded organelles was apparent. The sarcomeres were well registered and uniform. The sarcomere length was the same in the resting and stimulated fibers ($2.5 \pm 0.5 \mu\text{m}$ at rest and $2.4 \pm 0.2 \mu\text{m}$ when stimulated, mean \pm SD).

The myosin lattice volume, K_v , was measured for one of the stimulated muscle fiber and was $2.68 \times 10^9 \text{ \AA}^3$. In resting muscle the K_v was $2.21 \times 10^9 \text{ \AA}^3$ (from Eisenberg and Mobley, 1975). Thus, swelling in the order of 20% had occurred in the fibrils.

T Systems and Sarcoplasmic Reticulum

Careful inspection at higher magnification of the junction between the T tubule and the sarcoplasmic reticulum (SR) was undertaken to look for structural changes which might account for excitation-contraction uncoupling.

The T system was intact and had a normal appearance in most cases (Fig. 3 *a-f*), but some tubules show examples of broken or folded membranes (Fig. 3 *g* and *h*). The membrane of the T system shows signs of injury in resting and in fatigued fibers, but the difference is statistically insignificant (Table II). The damage may, therefore, be ascribed to fixation artifact and not to the experimental protocol. Measurement of the minor diameter of the T system shows a slight increase in the stimulated fibers, but again, the mean is not significantly different from resting fibers.

The width between the outer cytoplasmic leaflets of the T and TC membranes is 110–120 \AA in all fibers. Tiny pillars can be seen crossing between the membranes in many triads (Fig. 4 *a* and *b*). In tubules sectioned longitudinally, the pillars are found in a row along the length of the T-TC interface with the pillar to pillar spacing of 200–300 \AA . The spacing is the same when the T tubules are sectioned in their transverse plane and thus a tetragonal array is suggested. A pillar spans the 110 \AA T-TC gap, but is not necessarily seen perpendicular to the membrane surfaces. The outer diameter of the pillar is $\sim 100 \text{ \AA}$ and the central electron-lucent zone is up to 50 \AA wide. These dimensions cannot be determined with great precision because the pillars are much smaller than the thickness of the Epon section, and several pillars may be superimposed in any one electron micrograph image. Furthermore, fixation artifacts become significant for such tiny structures of unknown composition. More studies will need to be done with a variety of fixation methods and with electron micrographs taken at many tilt angles before a three-dimensional diagram can be drawn.

The frequency of occurrence of the pillars was found to be significantly greater in triads which had been stimulated, Table II. Moreover, only one resting fiber had a value of pillar frequency approaching the mean value of the stimulated fibers, and only one stimulated fiber had a value close to the resting fiber population. It is interesting that the occurrence of pillars was not correlated with fatigue or sarcomere length, but only with stimulation.

The internal composition of the terminal cisternae appears to be changed in the stimulated fibers. The amorphous granular material is denser throughout the cistern and particularly dense in a discontinuous plaque underlying the TC membrane which faces the T system (See Figs. 3 and 4). Furthermore, the

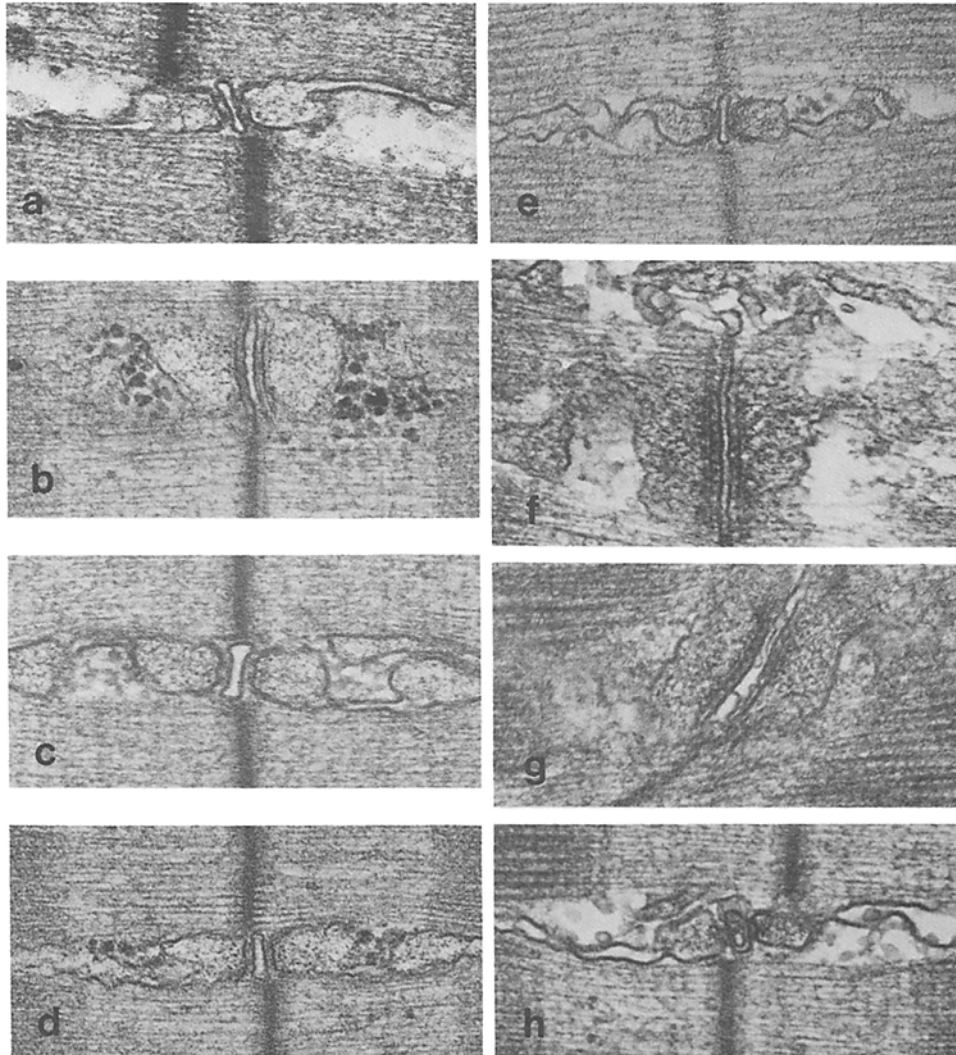


FIGURE 3. Electron micrographs of triads taken from fixed, longitudinally sectioned, single muscle fibers. (a-d) Normal, resting muscle; (e-h) fibers have twitched more than 300 times at 1 Hz. Note that stimulated triads have increased density in the terminal cisternae; (g and h), broken T tubules. Magnification $\times 60,000$.

percentage of terminal cisternae with dense plaques is related to the number of stimuli a fiber has received (see Fig. 5). Electron microscopic processing has many stages which could produce an increase in the absolute density of a printed micrograph. Therefore, some resting and stimulated single fibers were processed side-by-side (see Methods). In these experiments the density changes were also present in the stimulated but not in the resting fibers.

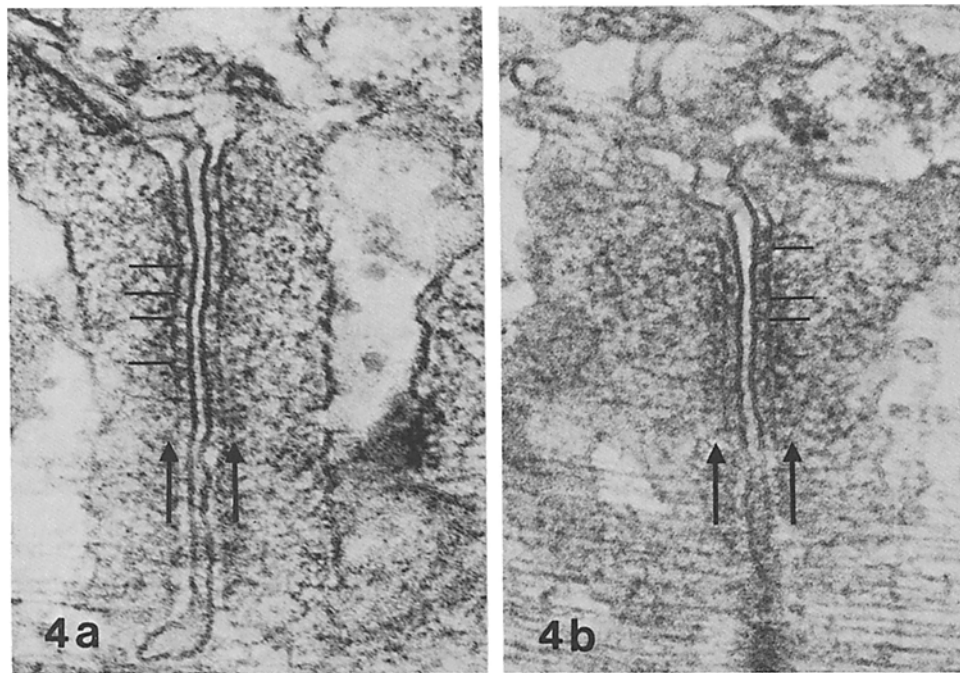


FIGURE 4. Electron micrographs of triads from stimulated fibers. Arrows, the dense plaque in the TC. Short lines, the connecting pillars. Magnification $\times 100,000$.

TABLE II
STRUCTURAL CHANGES IN THE T TUBULES AND
TERMINAL CISTERNAE PRODUCED BY STIMULATION OF
A MUSCLE FIBER AT 1 Hz

	T tubule diameter	T-TC gap	$\frac{\text{Broken T}}{\text{Total T}}$	$\frac{\text{Triads with pillars}}{\text{Total triads}}$
	Å		%	
Resting (n=6)	220±90	120±25	9±6	8±6
Early stage (n=4)	235±25	110±15	23±14	22±8
Fatigued (n=7)	270±50	110±30	13±12	21±13

Mean±SD.

Mitochondria

The mitochondria in resting muscle are elongated serpentine bodies lying between the myofibrils. They contain inner crista membranes packed like plates but with the angle of the plates to the fiber axis varying from one part of the mitochondria to the next (see Fig. 6 a-c). Thus, a micrograph generally shows

some crista membranes cut perpendicularly and the others cut tangentially. The width between adjacent perpendicular membranes was measured. The denser matrix region contains granules (See Figs. 2 and 6 *a-c*). In stimulated fibers, the granules are still present in approximately equal number and size (Table III). In that the volume of the mitochondria could have changed by some small but unmeasured amount, these values are not strictly comparable. The width between the inner crista membranes is unchanged whilst the matrix width increases substantially. Furthermore, it appears that the increase in the matrix width correlates with the total number of stimuli, but not with the fatigue index

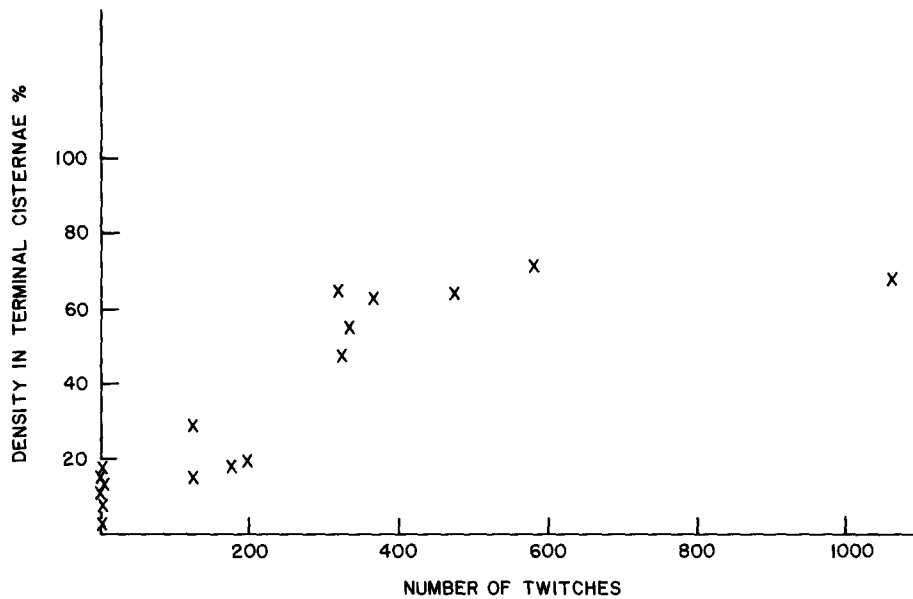


FIGURE 5. The percentage of TC with dense plaques as % total TC vs. number of twitches.

(Fig. 7). Because it is the innermost membrane compartment which is increasing in width, it follows that the outer mitochondrial membrane must have drastically altered shape towards the spherical or that the mitochondria must be swollen. The latter appears to be the case because the outer membrane sometimes appears broken or wrinkled (Fig. 6 *c*). In stimulated fibers, membranous blebs are found frequently on mitochondrial membranes as well as on the SR.

DISCUSSION

The purpose of this research is to determine structural alterations produced by low frequency stimulation to a single fiber. A quantitative evaluation of the subcellular structures has shown changes in the triadic junction, the myofibrils, and mitochondria.

The contractile filaments had the normal arrangement in stimulated fibers except for a slight increase in myosin-to-myosin spacing which results from fiber swelling. However, the cross bridges can still function at this distance as caffeine contractures are readily induced (Brust, 1976; Grabowski et al., 1972).

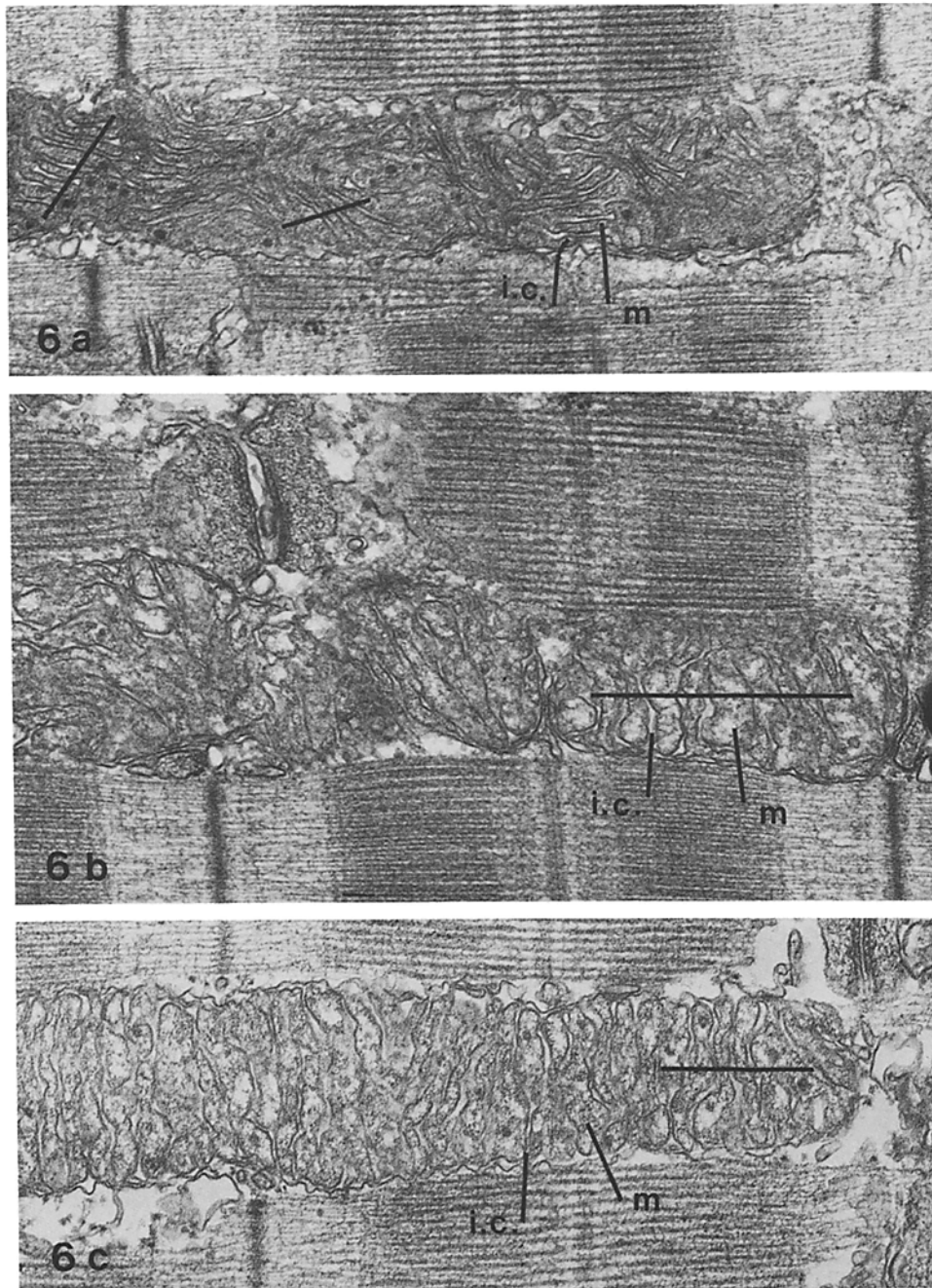


FIGURE 6. Electron micrographs of mitochondria from muscle fibers. Lines drawn perpendicular to cristal membranes used to measure matrix width (m) and inner cristae widths (i.c.). (a) Resting fiber; (b and c) fibers which have twitched more than 300 times at 1 Hz. Note granules are present in all fibers. Matrix widths are greater in stimulated fibers. Magnification $\times 42,000$.

Fatigue in rapidly stimulated fibers (30 Hz) has been attributed in part to vacuolization of the T system in tissue preserved by rapid freezing (Gonzalez-Serratos et al., 1978; Somlyo et al., 1978). Vacuoles can be seen in the living fibers with light microscopy (Krotenko, 1973), but none were apparent in our preparation. It is possible that some vacuoles could exist in fibers stimulated at low frequency and that chemical fixation failed to preserve them as in hypertonic muscle (Franzini-Armstrong et al., 1978). However, there are no significant remnants of broken or damaged tubules and only a slight increase in swelling of the T-tubule lumen. Therefore, it seems probable that the extensive damage noted by others is not responsible for the fatigue which we have measured. A few localized regions of T damage were seen, but if only a few T branches from the entire mesh fail, one would still expect the remainder of the

TABLE III
STRUCTURAL CHANGES IN THE MITOCHONDRIA
PRODUCED BY STIMULATION OF A MUSCLE FIBER

	Volume of granules	Diameter of granules	Number of granules
	Volume of mitochondria		Volume of mitochondria
	%	Å	μm^{-3}
Resting (n=6)	1.2±0.8	470±70	80±40
Early stage (n=4)	0.5±0.4	670±250	55±60
Fatigued (n=7)	0.5±0.5	600±110	30±20

Mean±SD.

T system to provide adequate activation. An electrophysiological study of fiber membrane capacitance during fatigue has not been reported, but this would be the method of choice to determine the integrity of the T system. However, the action potential shape changes in a manner consistent with an increased potassium conductance (Grabowski et al., 1972).

The gap between the T tubule and TC must be crossed by a functional mechanism which couples excitation of the T system and release of calcium from the TC. Although the gap width remained constant, the occurrence of the pillars was correlated with stimulation. Some published electron micrographs do show pillar-like structures in presumably resting fibers (Kelly, 1969, Fig. 5; Franzini-Armstrong, 1977, Fig. 3; and Somlyo et al., 1977, Fig. 5). A tannic acid fixation sometimes seems to enhance preservation of these structures in the resting muscle (Somlyo, 1979), but other times does not (Leeson, 1977). A report using freeze-fracture methods might suggest their anchorage to the membranes (Kelly, 1978). However, it is more notable that the pillars are not routinely seen with conventional fixation in resting fibers (Peachey, 1965; Eisenberg and Eisenberg, 1968; Franzini-Armstrong, 1977). Obviously more work must be done to establish their physiological significance, and cytochemistry is needed to determine their composition. It has not escaped our notice, however, that these

pillars might be analogous to the "rigid rods" invoked by Chandler et al. (1976) to provide a hypothetical mechanical link between the T system and the sarcoplasmic reticulum (Schneider and Chandler, 1973). It might also be possible for these pillars to provide a transient pathway for ionic current flow as invoked in the model of Mathias et al. (1979). However, the large molecule of horseradish peroxidase (50 Å) does not travel through this pathway during stimulation (Peachey and Eisenberg, 1978).

The increased density in the terminal cisternae is probably related to the increased calcium and phosphate concentrations seen by Somlyo et al., 1978. Excitation-contraction uncoupling cannot, therefore, be ascribed to the lack of

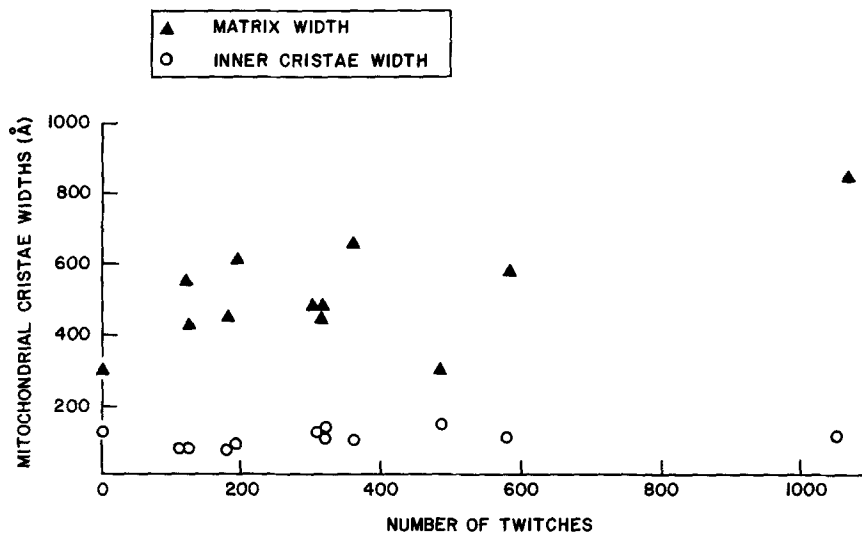


FIGURE 7. Relation between number of twitches and widths of the mitochondrial cristae.

calcium in the right place. Finally, the mitochondria do not cause fatigue because they have the normal appearance of an activated mitochondria with small granules (Goldstein et al., 1977). Failure of ATP production occurs when the mitochondria are swollen to their breaking point at which stage they accumulate large flocculent densities (Glaumann et al., 1977). However, ATP production clearly could be a rate-limiting step in some kinds of muscle fatigue.

Individual single fibers fatigued with different time-courses, and therefore, it was possible to determine which structural changes correlated with fatigue and which correlated with the total number of twitches. Our data show that no structural changes correlated to fatigue levels, and therefore, perhaps the mechanism of fatigue is at the molecular level. However, many structural changes were observed which could be correlated with the total number of stimuli given to a fiber, regardless of whether this resulted in fatigue or not. Stimulation-dependent changes were found in the mitochondrial cristae, the T-tubule shape and size, the increased density in the terminal cisternae, and

finally and perhaps of most interest, the increased number of pillars connecting the T system membrane to the TC membrane.

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