The usefulness of long fiber segments for the study of the pathophysiology of human skeletal muscle was evaluated. Immediately after biopsy, the fiber segments were depolarized. Within 3 hours the cut ends resealed, and if the segments were ≥2.5 cm long they regained normal resting membrane potentials (i.e., negative to −80 mV). Miniature endplate potentials, endplate potentials, action potentials, the current-voltage relationship, and the resting intracellular Ca^{2+} concentration of the resealed fiber segments were similar to those in fibers that were intact from tendon to tendon. In addition, specific properties of intact fibers obtained from patients with various neuromuscular diseases were preserved in the resealed fiber segments prepared from the same patients or patients with the same diseases. These segments are easily obtained as a routine muscle biopsy performed under local anesthesia; they provide valuable preparations for the study of the pathophysiology of human skeletal muscle as well as for in vitro pharmacological tests.
Key words: neuromuscular diseases • MEPPs • resting and action potentials • intracellular [Ca^{2+}] • myotonia • periodic paralysis • myasthenia gravis • malignant hyperthermia

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RESEALED FIBER SEGMENTS FOR THE STUDY OF THE PATHOPHYSIOLOGY OF HUMAN SKELETAL MUSCLE

FRANK LEHMANN-HORN, MD, MS, and PAUL A. IAIZZO, PhD

It is widely assumed that for the proper study of the physiology and pathophysiology of human skeletal muscle, fibers should be intact from tendon to tendon. Hence, the intercostal muscle is the only site from which intact fibers are routinely obtained from humans. The in vitro investigation of such fibers has been very useful for gaining insight into the pathophysiology of several muscle disorders.[8,17,21–23,25,26,29–31] Unfortunately, the intercostal biopsy normally re quires that the patient undergo general anesthesia and be hospitalized for a short period. If muscle fiber segments with one or two cut ends had properties similar to those of intact fibers, this would be very advantageous because such preparations could be more easily obtained, e.g., under local anesthesia from an arm or leg muscle. The study of fiber segments would be especially useful in the investigation of muscles from patients with progressive muscular dystrophy or myasthenia gravis. The commonly used methods of general anesthesia cannot be employed without placing such patients at risk.[7,28]

In this study, we describe the electrical and contractile properties of such muscle fiber segments in comparison with those of intact fibers. These preparations were obtained from normal subjects and patients with various neuromuscular diseases. This work was presented to a joint meeting of the German Physiological Society and The Physiological Society[15] and the 9th International Meeting on Neuromuscular Diseases in Marseille.[18]

PATIENTS

Bundles of muscle fiber segments were dissected from the latissimus dorsi, biceps brachii, deltoid,

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vastus medialis, or vastus lateralis muscles under local anesthesia with mepivacaine. In most cases, the segments were obtained from the motor point region of the muscle. We studied muscle fiber segments taken from normal subjects, from subjects susceptible to malignant hyperthermia, and from patients with the following neuromuscular diseases: recessive generalized myotonia (RGMy), Schwartz-Jampel syndrome (SJS), myotonic dystrophy (MyD), hyperkalemic periodic paralysis (HyperPP), hypokalemic periodic paralysis (HypoPP), and myasthenia gravis (MG). Additional data for some of these patients have been presented elsewhere.[9,16,19–21,30] The patients were chronologically numbered for easier cross-referencing between our reports.

Intact fibers were obtained from biopsies of the external intercostal muscle from patients with no known neuromuscular disorders who had to undergo thoracic surgery and also from several patients with known neuromuscular diseases (Table 1). In one patient with Schwartz-Jampel syndrome we were fortunate to obtain both intact fibers from the intercostal muscle and long fiber segments of the latissimus dorsi muscle during the same surgical procedure. In one patient with adynamia episodica hereditaria (or hyperkalemic periodic paralysis) intact intercostal fibers[21] and fiber segments of the biceps brachii were obtained on two different occasions. All procedures were in accordance with the Ethics Committee of the Technische Universität München and the Helsinki convention.

METHODS

The surgical removal of a specimen consisting of fiber segments was done with minimal stretch of the fibers. All transections were made with a scalpel. An excised specimen usually was ≥2.5 cm long. The cut ends of the fibers were not treated in any way. The specimen was placed into a dish with a sylgard bottom containing gassed solution at room temperature and mounted with several pins under slight stretch (10–20%). The specimen was dissected into thin bundles with diameters of 2–3 mm that were mounted in various experimental chambers with fine pins. For force measurements, one end of a bundle was attached with thread to a force transducer. Contractions and in vitro electromyographic activity were simultaneously recorded as described.[29] The experimental chambers were continuously perfused with gassed (95% O_2, 5% CO_2) solution.

Table 1. Resting membrane potentials (mV) of intact muscle fibers and long fiber segments from normal controls and patients with a neuromuscular disease.

Patient no.	Intact fibers	Long segments
RGMy1	−81.8 ± 4.4 (21)	—
RGMy2	−76.1 ± 14.8 (15)	—
RGMy4	—	−86.1 ± 9.3 (25)
RGMy5	—	−80.6 ± 7.7 (18)
RGMy6	—	−81.9 ± 4.8 (22)
RGMy7	—	−77.7 ± 5.1 (12)
SJS1	−85.3 ± 5.6 (28)	−83.2 ± 9.0 (13)
MyD10	—	−72.6 ± 9.2 (16)
MyD11	—	−66.4 ± 4.4 (8)
MyD12	—	−60.0 ± 2.9 (8)
MyD13	—	−82.7 ± 6.6 (20)
MyD14	—	−79.9 ± 8.7 (23)
HypoPP1	−71.2 ± 9.9 (48)	—
HypoPP2	−79.2 ± 5.3 (29)	—
HypoPP3	−77.1 ± 5.3 (29)	—
HypoPP4	−74.7 ± 9.0 (28)	—
HypoPP6	—	−66.1 ± 9.4 (47)
MG1	−81.9 ± 7.3 (21)	—
MG2	—	−76.5 ± 10.5 (4)
MH1	—	−46.4 ± 7.5 (16)*
MH2	—	−37.5 ± 5.9 (7)*
MH3	—	−79.5 ± 4.2 (4)
MH4	—	−80.5 ± 3.0 (6)
MH5	—	−78.9 ± 3.1 (16)
N1	−80.9 ± 4.9 (17)	—
N2	−78.4 ± 5.3 (18)	—
N3	−80.0 ± 5.1 (33)	—
N4	−83.5 ± 3.5 (14)	—

Values represent $\bar{X}$ ± *SD.*
() = number of fibers studied.
RGMy = recessive generalized myotonia.
SJS = Schwartz–Jampel syndrome.
MyD = myotonic dystrophy.
HypoPP = hypokalemic periodic paralysis.
MG = myasthenia gravis.
MH = malignant hyperthermia.
N = normal control.
**Segment lengths were between 2.5 and 3.0 cm.*

Electrical Properties. Resting membrane potentials (RMPs), miniature endplate potentials (MEPPs), and action potentials were recorded from the first layer of fibers with glass microelectrodes. The average MEPP amplitudes for each endplate were estimated from histograms of the amplitude distributions,[2] and only MEPPs with rise times <1.0 msec were used. For the study of endplate potentials (EPPs) the fibers were bathed in a solution containing alcuronium (0.7 μ*M*). The supplying nerve was stimulated with brief (0.07 msec) depolarizing current pulses by means of a glass suction electrode. In several experiments, after curare was removed from the bath, the stimulation of the nerve induced action potentials of the muscle fiber segments. Action potentials were

measured by an intracellular microelectrode which was shielded and its capacity compensated. A second microelectrode was impaled into the same fiber at a distance greater than 500 μm for applying a brief (0.1–0.5 msec) depolarizing pulse. In addition, the prestimulus potential could be adjusted to various levels by simultaneously adding constant current through this electrode. The protocol used to determine the *H*-infinitive curves was as previously reported.[21] For the measurement of the current-voltage relationship of the membrane, fibers were impaled midway with three microelectrodes as previously described.[22,30] All electrical measurements were performed at 37°C in a Bretag solution which had the same composition as that previously described.[22]

Statistical significance was determined by Scheffe's multiple contrast test (nonparametric).

Intracellular [Ca^{2+}]. The intracellular [Ca^{2+}] was determined by means of the fluorescent dye fura-2 in both intact fibers and long fiber segments (>2.5 cm). The mean fluorescence ratio, following excitation at 340 and 380 nm, was calculated. Using the mean ratio value of at least 10 fibers, the resting concentration was estimated by extrapolation from a calibration curve.[14] Force was simultaneously monitored throughout these measurements.

Electron Microscopy. Several hours after dissection, bundles of long fiber segments were immersion-fixed with phosphate-buffered 2.5% glutaraldehyde and embedded in Epon 812. Ultrathin sections were cut on an ultratome (LKB III, Munich, FRG), stained with lead citrate and uranyl-acetate, and examined with an electron microscope (Siemens Ia, Munich, FRG).

RESULTS

Resting Membrane Potentials (RMPs). *Dependency on Time.* Immediately after dissection of the small muscle bundles, all fiber segments were depolarized to values between −35 and −60 mV. Supramaximal electrical stimulation resulted in little or no contractile force. One hour after the dissection procedure, long fiber segments (≥2.5 cm) obtained from normal subjects had RMPs of −74 mV. At this time, the bundles were electrically excitable and thus capable of producing substantial force. Within the next 2 hours, the fiber segments completely repolarized to an average potential of −80 mV. These RMPs were stable for over 7 hours (Fig. 1A). The twitch tension produced following this degree of repolarization was comparable to that recorded from intact fiber preparations.

Long fiber segments from the patients with the various neuromuscular diseases also recovered to RMPs of approximately −80 mV. However, it should be noted that not all specimens from patients with myotonic dystrophy and from patients susceptible to malignant hyperthermia repolarized to normal values. In contrast, preparations from patients with recessive generalized myotonia appeared to recover faster and to a higher degree than all other preparations, including those from the normal subjects. The mean RMPs for both intact fibers and the long fiber segments obtained from the various patients and normal controls are listed in Table 1.

Dependency on the Segment Length. The segment length was directly related to the degree to which the fibers repolarized. Figure 1B shows the relative recovery of membrane potential for fiber segments of different lengths which were prepared from the same biopsied muscle. The time required for the initial repolarization was similar in all three bundles: 2 to 3 hours. However, at the end of this period, the average RMP was normal only in the longest fiber segments, and these potentials were stable for over 10 hours ($P < 0.01$). The somewhat large standard deviation reflects the fact that measurements from all fibers were included; even in intact preparations a certain percentage of the impalements are considered nonrepresentative.

Dependency on the Position of the Recording Electrode. Immediately after dissection of the muscle bundles, the fiber segments were depolarized along the entire length. This depolarization was more pronounced at the ends of the segments than in the central region where the following RMPs were recorded: about −60 mV in segments >2.5 cm; and about −40 mV in segments <2.0 cm. According to the equation for a cable with a length much greater than the space constant, one would expect quite normal RMPs of about −80 mV at regions several space constants away from the cut ends. The depolarization of the fiber segments may have resulted from damage of the fibers induced by the procedures of specimen removal and/or bundle dissection. Nevertheless, several hours after the biopsy the RMP along the entire length became nearly the same.

Additionally, it was observed that careful retransection at one end of long bundles, which were pinned in the experimental chamber, did not

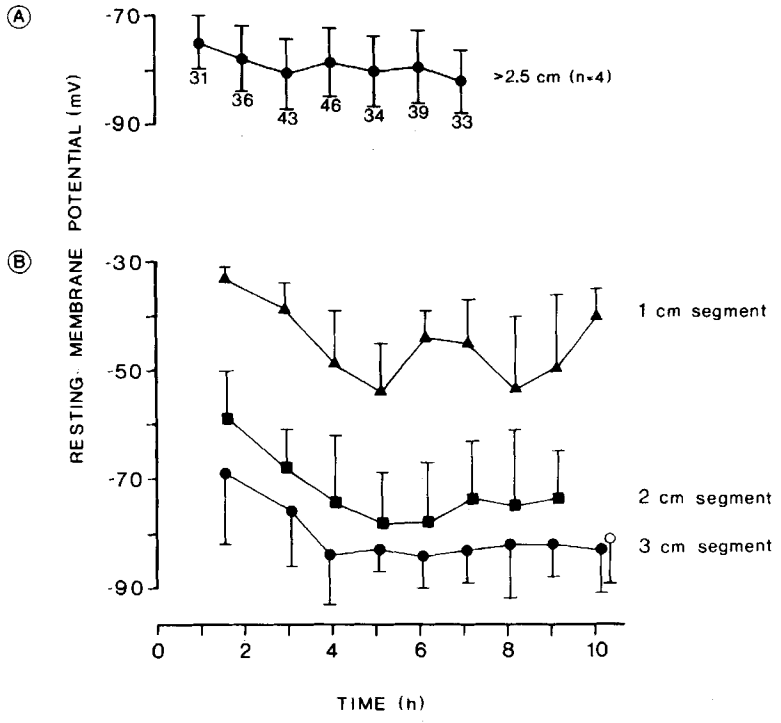

FIGURE 1. Effects of fiber transection and fiber length on resting membrane potential. The repolarization of fiber segments with time; resting membrane potentials in millivolts ($\bar{X} \pm$ SD). The fiber segments were randomly impaled. Only 19% of these had resting membrane potentials less negative than −65 mV: for the statistical analysis these values were not included **(A).** Three muscle bundles were prepared from the same muscle **(B).** One bundle had the same length as the whole biopsy, 3.0 cm (●). Another was recut to a length of 2.0 cm (■), and a third was recut to a length of 1.0 cm (▲). Ten hours after the biopsy the bundle which was 3.0 cm long was recut to a new length of 2.2 cm (○).

cause depolarization along the entire length of the fibers as did the origin biopsy procedure (Fig. 1B). The RMP in the middle of the fiber segments was unaltered. In this case, the depolarization of the fiber segments appeared to follow that which would have been predicted by the cable equation.[25]

MEPPs and EPPs. The amplitudes of MEPPs and EPPs are reported to be proportional to the RMP.[5] Depolarization of muscle fibers causes a decrease in the amplitude of endplate currents.[11] Fiber segments from normal controls which had repolarized to normal resting potentials had normal MEPPs and EPPs. Moreover, the average MEPP amplitude recorded from muscle fiber segments which were obtained from a patient with myasthenia gravis and which had fully repolarized was significantly lower than in normal fiber segments. On the other hand, this patients's values did not differ from the average MEPP amplitude recorded in intact fibers from another myasthenia patient (Table 2).

In the presence of 0.7 μM alcuronium (Alloferin®), EPPs were elicitable. At a given RMP, the amplitude of the EPPs varied in a steplike fashion. This was indicative of a different number of quanta (acetylcholine) being released. The reversal potential in both types of preparations was estimated to be between 0 and −10 mV. At a holding potential of −80 mV the relative amplitudes of the EPPs recorded from the intact fibers and the resealed segments were the same ($P >$ 0.25). As curare was removed from the bath, action potentials were induced by nerve stimulation.

Action Potentials. The action potentials were similar in the intact fibers and the resealed fiber segments from normal muscles. Constant current was added to alter the RMP (−70 to −120 mV, in 10 mV steps). Action potentials elicited at these prestimulus potentials in the intact and resealed fibers had maximum rates of rise and peak potentials (Table 2) which were not significantly different ($P > 0.25$). Hence, intact fibers and the fiber segments had similar H-infinitive curves. In contrast, action potentials recorded from fibers obtained

Table 2. Electrophysiological properties of intact and resealed fibers from normal subjects and those with various neuromuscular diseases.

	Normal controls		Patients with various neuromuscular diseases		Patient ID
Electrophysiological property	Intact	Segment	Intact	Segment	
Membrane resistance R_m ($cm^2 \times 1000$)	4.9 ± 1.2 (9)*	3.8 ± 0.7 (10)*	8.6 ± 4.2 (16)†	11.7 ± 4.0 (10)	RGMy1,7
Membrane conductance g_m ($S/cm^2 \times 100$)	2.2 ± 0.6 (9)*	2.7 ± 0.4 (10)*	1.3 ± 0.4 (16)	0.9 ± 0.2 (10)	RGMy1,7
MEPP amplitude (mV)	0.43 ± 0.15 [9]	0.36 ± 0.03 [18]	0.15 ± 0.03 [6]	0.24 ± 0.11 [7]	MG1,2
Action potentials overshoot potential (mV)	12.9 ± 8.0 (21)	6.0 ± 5.0 (16)	−7.8 ± 4.8 (10)	−9.1 ± 8.0 (8)	HypoPP5,6
Max. rate of rise (V/s × 100)	3.7 ± 1.2 (17)	4.0 ± 0.5 (21)	2.1 ± 0.3 (10)	1.8 ± 0.5 (8)	HypoPP5,6

Values represent $\bar{X}$ ± SD.
() = number of fibers studied.
[] = number of endplates.
RGMy = recessive generalized myotonia.
HypoPP = hypokalemic periodic paralysis.
MG = myasthenia gravis.
**Extracellular [K^+] of 4.5 mM.*
†Values from Rüdel et al.[31]

from patients with hypokalemic periodic paralysis were significantly different from those of the normal fibers. The rates of rise were slower than the normals, and an overshoot did not occur (Fig. 2A). On the other hand, there was no difference between action potentials recorded from either intact fibers or fiber segments from the various patients who had hypokalemic periodic paralysis (e.g., Table 2). Finally, it was possible to record spontaneous runs of action potentials in long fiber segments from either patients with recessive generalized myotonia or myotonic dystrophy (Fig. 2B and C).

Current-Voltage Relationship. There was no difference between the current-voltage relationships derived from intact fibers and fiber segments from normal muscle. The current-voltage relationships derived from fiber segments from patients with recessive generalized myotonia (e.g., Fig. 3) had a slope that was less steep in the range of the resting potential. This behavior was identical to that previously described for intact fibers from another patient,[31] which reflects a lower membrane (chloride) conductance (Table 2).

Force Measurements. For over 8 hours, reproducible twitches and tetani could be recorded from bundles of long fiber segments (≥2.5 cm). Preparations from patients with a myotonic disorder displayed after-activity following stimulation. There was a good correlation between the electrical after-activity and the slowed relaxation (Fig. 4). If the bundles of fiber segments from these patients were short, and thus the membranes were depolarized, upon supramaximal stimulation they produced little force, and the recorded after-activity was minimal. For 5 hours reproducible tetani could be elicited in long fiber segments from a patient with hyperkalemic periodic paralysis. Figure 5 shows the effects of elevated extracellular K^+ and low pH on these resealed fibers. The responses were the same as described for intact intercostal fibers obtained from this patient.[21]

Intracellular [Ca^{2+}]. For normal muscle, the resting intracellular [Ca^{2+}] of long fiber segments was not significantly different ($P > 0.25$) from that estimated for intact fibers (Fig. 6). According to our calibration measurements,[14] both values were approximately 0.10 μM. In contrast, both long fiber segments of the latissimus dorsi and intact intercostal fibers obtained from the same patient with Schwartz-Jampel syndrome were found to have elevated intracellular [Ca^{2+}] at rest (Fig. 6). The elevated [Ca^{2+}] was not related to excessive spontaneous activity or trauma for: (1) the preincubation of the fiber segments in TTX for over 1 hour did not alter these levels, and (2) both the intact fibers and the fiber segments from this patient had normal RMPs before and after the fluorescence measurements. The resting intracellular [Ca^{2+}] in these fiber segments was estimated to be 0.24 μM.

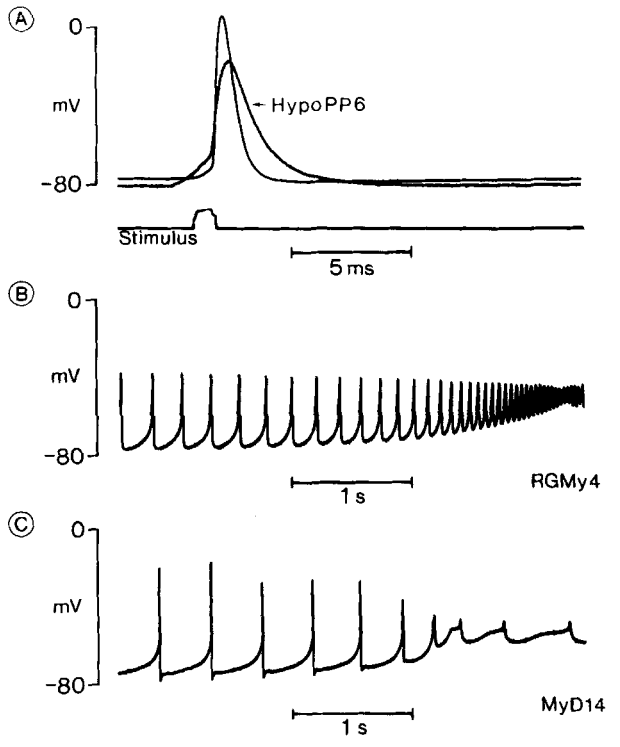

FIGURE 2. Action potentials recorded from long fiber segments. **(A)** A normal action potential and that from a patient with hypokalemic periodic paralysis (HypoPP6). **(B)** A spontaneous run of action potentials recorded from a fiber segment obtained from a patient with recessive generalized myotonia (RGMy4). **(C)** A spontaneous run of action potentials recorded from a fiber segment obtained from a patient with myotonic dystrophy (MyD14). The prepotentials in each of these records were approximately −80 mV.

Electron Microscopy. During the biopsy procedure the specimen contracted and thus it was shorter than in situ. Subsequently, the fiber segments relaxed, except for their very ends (1–2 mm), where the myofibrils remained hypercontracted. Because of this sustained shortening of distal myofibrils, the sarcolemma and the basal membrane were long enough to enclose the cut ends and to reseal (Fig. 7).

DISCUSSION

Transection of a muscle fiber causes an active shortening of each of the resulting fiber segments. This in part may be due to (1) an initial propagated depolarization of the excitable membrane (contraction), (2) a long-lasting membrane depolarization (contracture), and/or (3) the direct influx of Ca^{2+} into the intracellular space at the cut ends (hypercontracted band).

It has been well documented that skeletal muscle fibers depolarize when transected. As early as the nineteenth century DuBois-Raymond[6] described a "Verletzungspotential" of muscle fibers, which means a potential created by "hurting" or "damaging" the fiber. In addition, several authors have reported the use of cut muscle preparations to obtain "physiological" EPPs.[1,13,34] In these reports intact fibers were cut to reduce the RMP to levels at which most Na^+ channels were inactivated and action potentials could not be elicited or propagated. Thus these fiber segments were electrically inexcitable and therefore paralyzed.

On the other hand, segments of muscle fibers have been used extensively for the "in vitro contracture test" to determine susceptibility to malignant hyperthermia (for a review, see Ørding[27]). This test determines the sensitivity of cut muscle fibers to caffeine or halothane applied to the bathing solution. Fiber segments from persons susceptible to malignant hyperthermia have lower contracture thresholds for these agents than those from normal subjects. Although this test is in frequent use, little was known about the physiological state of transected fibers. To our knowledge, there is only one report in which the electrical properties of fiber segments were investigated as a method for studying neuromuscular diseases, that of Uchitel and Dubrovsky.[33] In their study, they reported that the RMPs of the fiber segments

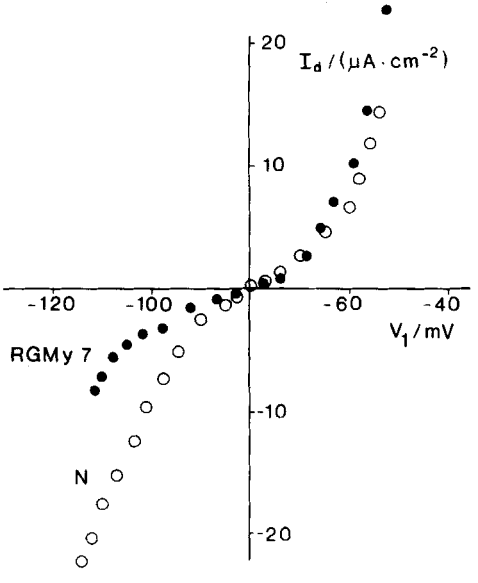

FIGURE 3. Current-voltage relationships of long fiber segments from normal fibers (n =(○)) and those from a patient with recessive generalized myotonia (n =) (RGMy7). These curves are similar to those reported for intact fibers.[31]

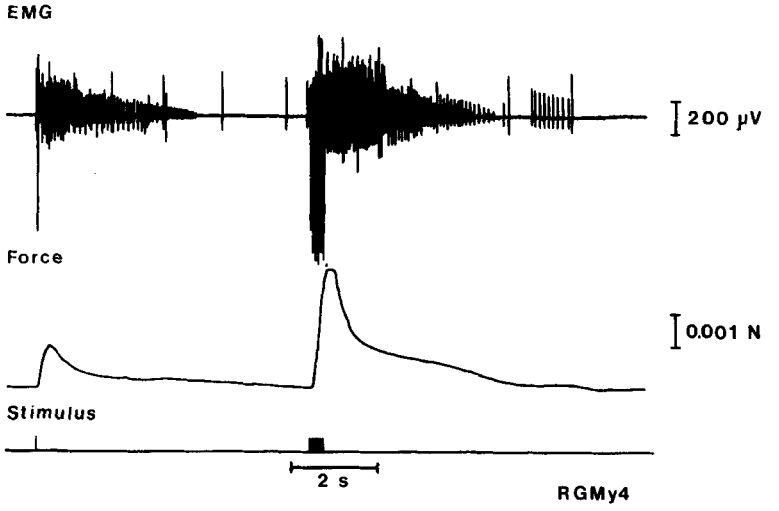

FIGURE 4. Electrical and mechanical after-activity recorded from fiber segments obtained from a patient with recessive generalized myotonia (RGMy4). The resting membrane potentials of the fiber segments were normal (−80 to −90 mV) prior to stimulation with either a single pulse or a short (300 msec) tetanic train (30 Hz).

ranged from −30 to −70 mV. They stated that the lengths of the cut fibers they studied were between 2 and 3 cm long. This appears to be somewhat in contrast to what we observed. We report here that fibers with a length of approximately 2.5 cm or longer, in time, can repolarize to normal potentials of −80 to −90 mV. Nevertheless, we do agree with their suggestion that fiber segments are suitable for electrophysiological studies.

What is the reason for the depolarization induced by the transection? The K^+ gradient across the membrane of a fiber segment does not appear to be drastically abnormal prior to recovery of normal RMPs. In a related study, it has been shown that a drug which opens K^+ channels (cromakalim) readily hyperpolarized fiber segments which had not yet fully recovered.[32] The fact that the fibers reseal and do not loose large quantities of K^+ may explain why in patients undergoing

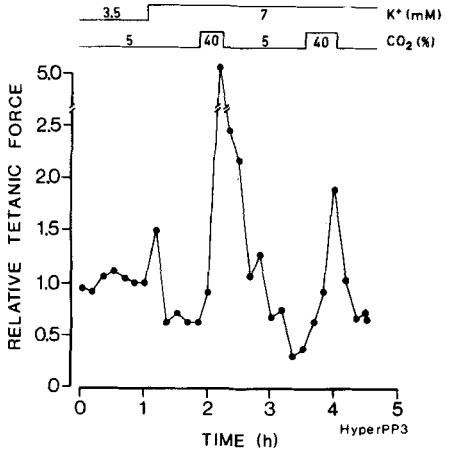

FIGURE 5. The effects of elevated K^+ and low pH on tetanic contractions of fiber segments from a patient with hyperkalemic periodic paralysis (HyperPP3). The tetanic force (30 Hz stimulation of 300 msec duration) decreased when the extracellular [K^+] was raised and increased again when the pH of the high-K^+ solution was lowered by gassing the solution with a mixture of 40% CO_2 and 60% O_2.

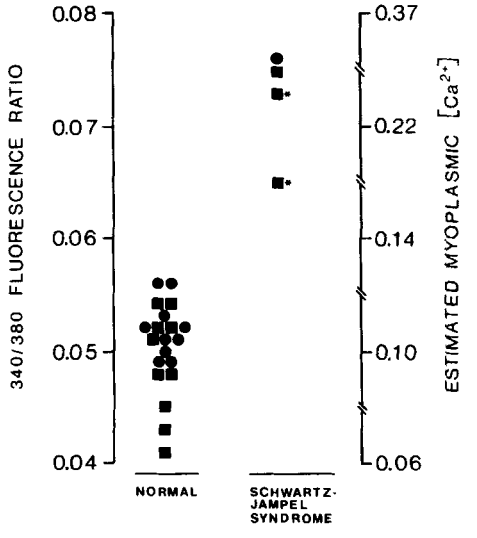

FIGURE 6. Mean fura-2 fluorescence ratios and estimated free intracellular [Ca^{2+}] of long fiber segments (■) and intact fibers (●) under control conditions. The preparations were obtained from normal subjects and from one patient with Schwartz-Jampel syndrome. Two of the bundles of resealed fiber segments were preincubated in 1 μ*M* tetrodotoxin (* = TTX) to eliminate any possible spontaneous activity.

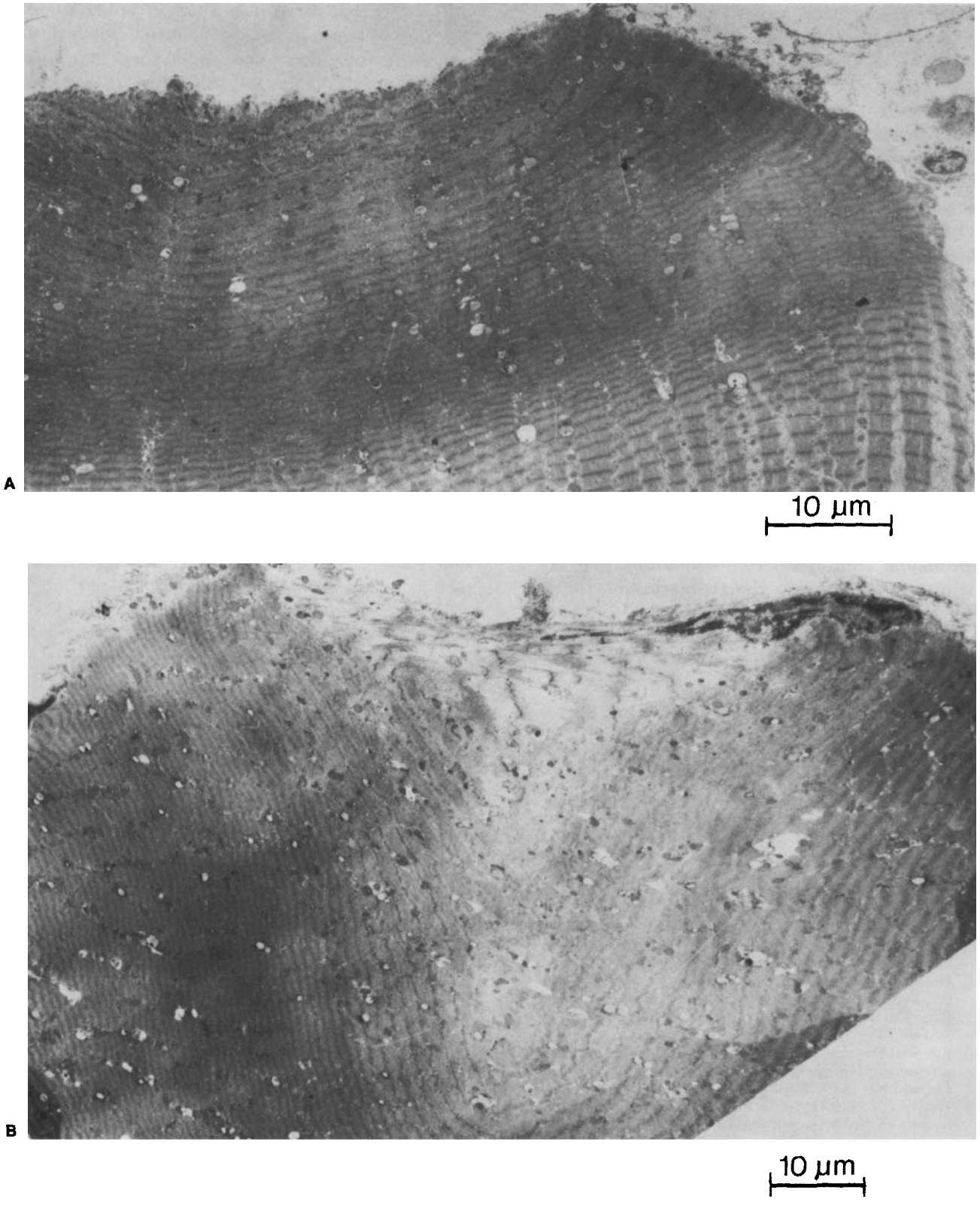

FIGURE 7. Electron micrographs of the cut end regions of fiber segments from a normal subject. The hypercontracted regions extended for 1–2 mm (e.g., as in **A**). In **B,** the hypercontraction formed an indentation of the central myofibrils, and the cut sarcolemma resealed. The appearance of the sarcomeres beyond these hypercontraction zones was normal.

surgical procedures which required transection of large quantities of muscle, the serum [K^+] does not become abnormally elevated as might have been predicted.

Several hours were required for recovery of RMP in the absence of such a drug, and thus we hypothesize that it was the action of the ATP-dependent Na^+/K^+ pump which slowly lead to recovery of normal membrane potential. The action of the pump alone has been reported to alter the membrane potential by only 5–10 mV,[4] but this may be all that is required to cause other important events to occur, such as (1) the decreased activation of voltage-dependent Na^+ channels, or (2) the closing of some type of nonspecific stretch-activated channels which may have opened during the biopsy procedure.[3,12] The fiber segments appear to have an adequate supply of ATP for this mechanism to function, because such preparations can develop stable mechanical responses for hours following repolarization and possess normal intracellular [Ca^{2+}] under resting conditions. Likewise, in support of this hypothesis, we observed that adrenaline (unpublished observation) was often helpful in accelerating the repolarization process: adrenaline has been reported to stimulate the Na^+/K^+ ATPase pump.[4] If fiber segments are too short, then there may remain too little intact membrane available for recovery to occur. It should be noted that, also in preparations of fibers intact from tendon to tendon, often the fibers are initially (immediately after dissection) somewhat depolarized but will recover to RMPs more negative than −80 mV within a few hours (a similar time period required by the fiber segments). It should also be noted that the ability of fiber segments from patients with myotonic dystrophy and patients susceptible to malignant hyperthermia to repolarize seems to be less than in other preparations. This inability to repolarize completely may be related to their reported higher injury sensitivities.[10,14,24]

Only nerve axons were thought to recover following transection and be able to propagate action potentials as before. But muscle fiber segments can become fully excitable as well. Resealed fiber segments which repolarized to normal RMPs were observed to have the same properties as in vivo, which is not always true for cultured muscle cells such as myotubes and/or myoballs.

REFERENCES

1. Barstad JAB: Presynaptic effect of neuro-muscular transmitter. *Experientia* 1962;18:579–580.
2. Boyd IA, Martin AR: Spontaneous subthreshold activity at mammalian neuromuscular junctions. *J Physiol* 1956;132:61–73.
3. Brehm P, Kullberg R, Moody-Corbett F: Properties of nonjunctional acetylcholine receptor channels on innervated muscle of Xenopus laevis. *J Physiol* 1984;350:631–648.
4. Clausen T, Flatman JA: The effect of catecholamines on Na-K transport and membrane potential in rat soleus muscle. *J Physiol* 1977;270:383–414.
5. Cull-Candy SG, Miledi R, Trautmann A: End-plate currents and acetylcholine noise at normal and myasthenic end-plates. *J Physiol* 1979;287:247–265.
6. Du Bois-Raymond E: *Untersuchungen über thierische Elektrizität.* Reimer, Berlin, 1848.
7. Ellis FR: Neuromuscular disease and anaesthesia. *Br J Anaesth* 1974;46:603–612.
8. Elmqvist D, Johns TR, Thesleff S: A study of some electrophysiological properties of human intercostal muscle. *J Physiol* 1960;154:602–607.
9. Franke Ch, Hatt H, Iaizzo PA, Lehmann-Horn F: Characteristics of Na^+ channels and Cl− conductance in resealed muscle fibre segments from patients with myotonic dystrophy. *J Physiol,* to be published.
10. Gallant EM, Fletcher TF, Goettl VM, Rempel WE: Porcine malignant hyperthermia: cell injury enhances halothane sensitivity of biopsies. *Muscle Nerve* 1986;9:174–184.
11. Glavinovic MI: Voltage clamping of unparalysed cut rat diaphragm for study of transmitter release. *J Physiol* 1979;290:467–480.
12. Guharay F, Sachs F: Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J Physiol* 1984;352:685–701.
13. Hubbard JI, Wilson DF: Neuromuscular transmission in a mammalian preparation in the absence of blocking drugs and the effect of d-tubocurarine. *J Physiol* 1973;228:307–325.
14. Iaizzo PA, Klein W, Lehmann-Horn F: Fura-2 detected myoplasmic calcium and its correlation with contracture force in skeletal muscle from normal and malignant hyperthermia susceptible pigs. *Pflügers Arch* 1988;411:648–653.
15. Iaizzo PA, Lehmann-Horn F: Long cut muscle fibers repolarize and possess electrical and mechanical properties of intact human skeletal muscle. *Pflügers Arch* 1988;411:R191.
16. Iaizzo PA, Lehmann-Horn F: The correlation between electrical after-activity and slowed relaxation in myotonia. *Muscle Nerve,* 1990;13:240–246.
17. Kwiecinski H, Lehmann-Horn F, Rüdel R: The resting membrane parameters of human intercostal muscle at low, normal, and high extracellular potassium. *Muscle Nerve* 1984;7:60–65.
18. Lehmann-Horn F, Iaizzo PA: Use of fiber segments to investigate the pathophysiology of human skeletal muscle, in Serratrice G, Pellissier J-F, Desnuelle C, Pouget J (eds.): *Myélopathies, neuropathies et myopathies.* Expansion Scientifique Française, Paris, 1989, pp 44–50.
19. Lehmann-Horn F, Iaizzo PA: Are myotonias and periodic paralyses associated with susceptibility to malignant hyperthermia? *Br J Anaesth,* to be published.
20. Lehmann-Horn F, Iaizzo PA, Franke C, Hatt H, Spaans F: Schwartz-Jampel syndrome. Part II: Na^+ channel defect causes myotonia. *Muscle Nerve,* 1990; to be published.
21. Lehmann-Horn F, Küther G, Ricker K, Grafe P, Ballanyi K, Rüdel R: Adynamia episodica hereditaria with myoto-

nia: a noninactivating sodium current and the effect of extracellular pH. *Muscle Nerve* 1987;10:363–374.
22. Lehmann-Horn F, Rüdel R, Dengler R, Lorkovic H, Haass A, Ricker R: Membrane defects in paramyotonia congenita with and without myotonia in a warm environment. *Muscle Nerve* 1981;4:396–406.
23. Lehmann-Horn F, Rüdel R, Ricker K: Membrane defects in paramyotonia congenita (Eulenburg). *Muscle Nerve* 1987;10:633–641.
24. Lipicky RJ: Studies in human myotonic dystrophy, in Rowland LP (ed): *Pathogenesis of Human Muscular Dystrophy.* Amsterdam, Excerpta Med, 1977, pp 729–738.
25. Lipicky RJ, Bryant SH, Salmon JH: Cable parameters, sodium, potassium, chloride and water content, and potassium efflux in isolated external intercostal muscle of normal volunteers and patients with myotonia congenita. *J Clin Invest* 1971;50:2091–2103.
26. Mora M, EH Lambert, Engel AG: Synaptic vesicle abnormality in familial infantile myasthenia. *Neurology* 1987;37:206–214.
27. Ørding H: Diagnosis of susceptibility to malignant hyperthermia. *Br J Anaesth* 1988;60:287–302.
28. Ravin M, Newmark Z, Saviello G: Myotonia dystrophia—an anesthetic hazard: two case reports. *Anesth Analg* 1975;54:216–218.
29. Ricker K, Rüdel R, Lehmann-Horn F, Küther G: Muscle stiffness and electrical activity in paramyotonia congenita. *Muscle Nerve* 1986;9:299–305.
30. Rüdel R, Lehmann-Horn F, Ricker K, Küther G: Hypokalemic periodic paralysis: in vitro investigations of muscle fiber membrane parameters. *Muscle Nerve* 1984;7:110–120.
31. Rüdel R, Ricker K, Lehmann-Horn F: Transient weakness and altered membrane characteristic in recessive generalized myotonia (Becker). *Muscle Nerve* 1988;11:202–211.
32. Spuler A, Lehmann-Horn F, Grafe P: Cromakalim (BRL 34915) restores in vitro the membrane potential of depolarized human skeletal muscle fibres. *Naunyn-Schmiedeberg's Arch Pharmacol* 1989;339:327–331.
33. Uchitel OD, Dubrovsky AL: Electrophysiologic denervation changes of human muscle fibers in motoneuron diseases. *Muscle Nerve* 1986;9:748–755.
34. Wilson DF: Estimates of quantal-release and binomial statistical-release parameters at rat neuromuscular junction. *Am J Physiol* 1977;233:C157–C163.