MUSCLE CONTRACTION GENERATES DISCRETE SOUND BURSTS

FRANK V. BROZOVICH AND GERALD H. POLLACK
Department of Anesthesiology and Division of Bioengineering, University of Washington, Seattle, Washington 98195

ABSTRACT Isolated frog sartorius muscles were stimulated to shorten under lightly loaded conditions. A piezoelectric transducer was placed alongside the muscle to record sounds generated during contraction. Shortening was accompanied by the generation of a series of discrete sound bursts. The bursts were found to be moderately repeatable among successive contractions; 44% repeated from contraction to contraction. The duration of each sound burst was on the order of 400 μs, and the temperature dependence of the interval between successive bursts had a Q_{10} of ~2. Sound intensity was variable: average acoustic power ranged from 0.05–0.4 mW/g, or ~1% of the heat generated during contraction. The generation of discrete bursts of sound during contraction, rather than continuous sound, implies that contractile behavior may be discontinuous.

INTRODUCTION

That contracting muscles generate sounds has been known for almost two centuries (Wollaston, 1810). Using a stethoscope placed over a contracting muscle in vivo, several investigators (Wollaston, 1810; Herroun and Yeo, 1885; Oster and Jaffe, 1980) have recorded continuous, low-frequency sounds. Others (Gordon and Holbourn, 1948), using equipment with a wider frequency response, have reported that such sounds are generated as discrete spikes.

We assumed that the sounds could be more faithfully discerned from an isolated muscle shortening in vitro, using a sensitive, broad-band transducer. In such a preparation, sounds would not be distorted by surrounding tissues, and their character could be more reliably ascertained. If recorded sounds were indeed discrete, not continuous, this could imply that the contractile process is also discrete.

METHODS

Experimental Setup

Sartorius muscles of *Rana pipiens*, *Rana temporaria*, or *Osteopilus septentrionalis* (Cuban tree frogs) were mounted in a sound-insulated muscle chamber (Fig. 1). To absorb sounds and prevent reflections, the chamber was lined with neoprene rubber, a common acoustic insulator (absorption coefficient = 0.80 at 2 kHz; Nolle and Mowry, 1948).

One tendon was tied with surgical thread and connected to a force transducer with a frequency response of 300 Hz (Statham UC-2, Gould Inc. Meas. Sys. Div., Oxnard, CA). A hole was placed in the other tendon, which was hooked onto a lever to allow lightly loaded contraction. The muscle was bathed in a physiological saline solution with the following millimolar concentrations: [Mg^{2+}], 1.0; [Na^+], 101; [K^+], 2.5; [Cl^-], 108; [Ca^{2+}], 1.8; [$H_2PO_4^-$], 1.0; [imidazole], 20. Glucose was added to a final concentration of 0.9 g/liter and the solution was titrated to pH 7.8. The solution was cooled to the desired temperature, ranging from 0°–15°C, in an ice bath, and was renewed about every 30 min.

A piezoelectric sound transducer was placed 0.5–1.0 cm from the muscle. The transducer consisted of a hemispherical (1.2 cm diameter), ceramic (PZT-5) crystal, which resonated in a radial expansion mode (Marine Resources, Inc., Fern Park, Florida). The crystal was mounted in an air-backed housing so that its axis of symmetry was perpendicular to the long axis of the muscle.

The outputs from the sound and tension transducers were amplified and the sound output was band-pass filtered from 500 Hz to 250 kHz to eliminate from the system low- and high-frequency noise that derived predominately from aberrant mechanical vibrations and electrical noise. Filtering did not noticeably alter the characteristics of the recorded sound spikes. Sound and force records were sampled every 100 μs and displayed on a digital storage oscilloscope (Gould Inst. Div., Biomation, model 820). In a single experiment, a sampling interval of 10 μs was used. The recorded sound spikes were found to have a duration of ~400 μs, similar to the results found with the sampling interval of 100 μs. The higher sampling rate was not used because limited storage capacity precluded storage of data throughout the entire twitch. Records were photographed.

Experimental Procedure

A single stimulus pulse of 1 ms duration was applied every 6 s through a pair of electrodes parallel to the long axis and slightly beneath the muscle. To assure that all the fibers were excited, the amplitude of the stimulus was raised until no additional force was developed during shortening. Under the lightly loaded conditions (nearly zero load) used here, shortening of 20–30% was observed. The muscle was aligned in the chamber by rotating one of its tendons so contraction could proceed without any visually detectable twist. Muscles were not appreciably stretched by the system. The dimensions of several specimens were measured with a stereo microscope. Their length was 30 ± 1 mm (n = 4), and the major and minor axes of their cross section were 5 ± 1 mm and 2 ± 1 mm.

In a preliminary investigation on a group of 53 muscles, it was found that shortening often was accompanied by the generation of sound spikes (Brozovich and Pollack, 1981). This study reports the results obtained from an additional 74 muscles using the improved measurement techniques described herein. The muscles had been randomly assigned to three experimental groups: 39 were used to elucidate the temperature dependence of the sound bursts, 23 were studied in sound repeatability

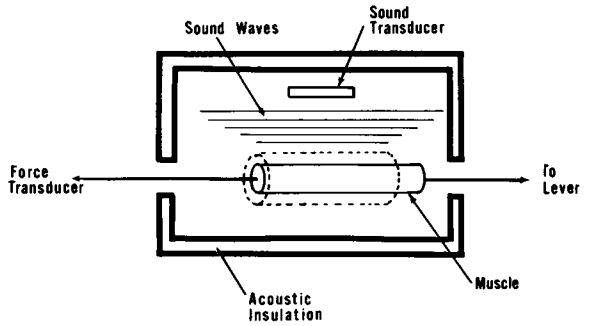

FIGURE 1 Experimental apparatus. Also shown, diagramatically, are the width changes expected to accompany muscle shortening. Rapid changes of cross-sectional area should generate sound bursts, which may then be detected by the piezoelectric sound transducer.

experiments, and 12 were used to determine the main frequency component of the bursts.

Characterization of the Sound Transducer

To optimize its sensitivity, the piezoelectric crystal was mounted in a transducer housing with an air backing. Backing materials affect the resonance characteristics of a piezoelectric crystal (Martin and Sigelmann, 1975); therefore, the frequency-impedance relationship of the transducer was determined both in air (unloaded) and in water (loaded) using a vector voltmeter (Hewlett-Packard Co., Palo Alto, CA, model 4815A). The air-backed transducer was resonant at 899 kHz without a load and the water-loaded transducer resonated at 900 kHz.

From the frequency-impedance characteristics, the relative efficiency of conversion of sound pressure to electrical voltage was computed as an attenuation factor (Martin, 1977):

$$\text{attenuation factor} = \frac{R_{H_2O}}{R_{H_2O} + R_{AIR}}$$

where, R_{H_2O} is the real part of the transducer's impedance in water, and R_{AIR} is the real part of the transducer's impedance in air. Using the radiation force balance method (Rooney, 1973), the sensitivity was determined by exciting the transducer at its resonant frequency with a known input voltage. The acoustic force generated by the transducer was coupled to one arm of a balance (cf. Rooney, 1973, for details) and measured. The calibration curve (mW/V vs. frequency) was obtained by dividing the sensitivity, calculated at resonance, by the attenuation factor at each frequency (Fig. 2).

The power recorded was a fraction of the total power radiated from the muscle. Assuming a uniform pattern of radiation, this fraction is determined by the ratio of the solid angle subtended by the transducer to the solid angle of the sphere surrounding the muscle. With the geometry of the present setup, this ratio was 0.03. Thus, from Fig. 2, the sensitivity of the system below 0.7 MHz to the actual energy generated by the muscle was 14 mW/V. All results are expressed in these terms.

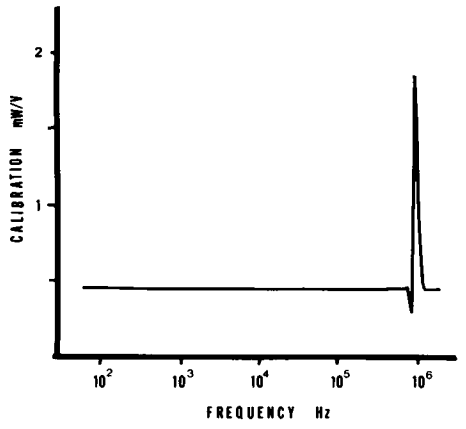

FIGURE 2 Response of the transducer to acoustic energy (mW/V) as a function of frequency.

RESULTS

Discrete sound bursts were recorded during contraction in 49 of the 74 muscles. In 14 muscles, a single sound spike was detected during shortening or lengthening while in the remaining 35, twitches were accompanied by a series of sound spikes (Fig. 3).

The pattern of sound bursts was moderately repeatable from contraction to contraction in the same muscle (Fig. 4). To be considered repeatable, a spike had to occur within ± 4 ms (chosen for convenience in analysis) of a sound spike in a previous contraction. Using this criterion, we found that 44% of the bursts repeated. Others disappeared and were sometimes replaced by new spikes occurring at a different time.

The amplitudes of the bursts were variable. No spikes were counted if their amplitude was <1 mW because the noise level was occasionally that high. The intensity of the accepted sounds, thus, ranged from 1.1 to 3.8 mW. The histogram of burst energy peaked near 2.0 mW (Fig. 5).

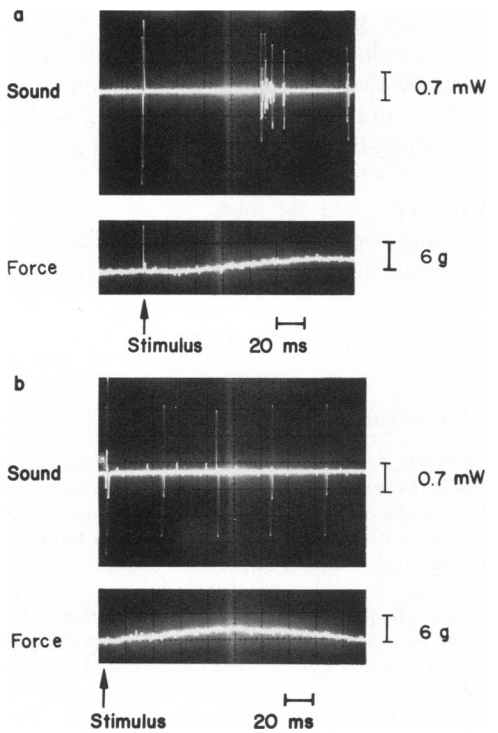

FIGURE 3 Two examples of a series of discrete sound bursts recorded from twitching sartorius muscles. The force record is displayed and the stimulus artifact is labeled.

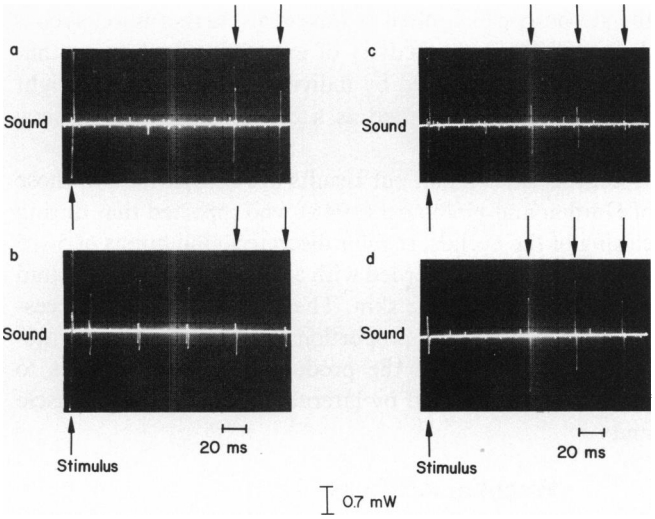

FIGURE 4 Sound bursts recorded from successive twitches of the same muscle; (*a*) and (*b*) muscle 1, (*c*) and (*d*) muscle 2. Note that some bursts are repeatable as indicated by the arrows.

In 12 experiments, the sound bursts were stored on the digital oscilloscope and their time scale was expanded to determine their period (Fig. 6). The period of 19 sound spikes generated by 7 different muscles was measured and found to be 400 ± 5 μs ($\bar{x}$ ± SD). The accuracy was limited by the ability to measure the spike period. A sampling frequency of 10 kHz allows frequency components up to 5 kHz to be accurately determined (Oppenhein and Schafer, 1975). As a check that this frequency was not an artifact of the transducer's resonance characteristics, the period of

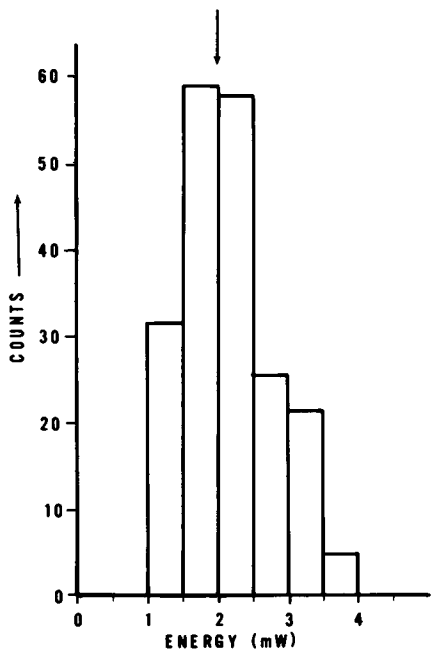

FIGURE 5 Histogram of sound burst amplitude. The peak is indicated by the arrow. No sound spikes were measured under 1 mW as the noise level was occasionally that high.

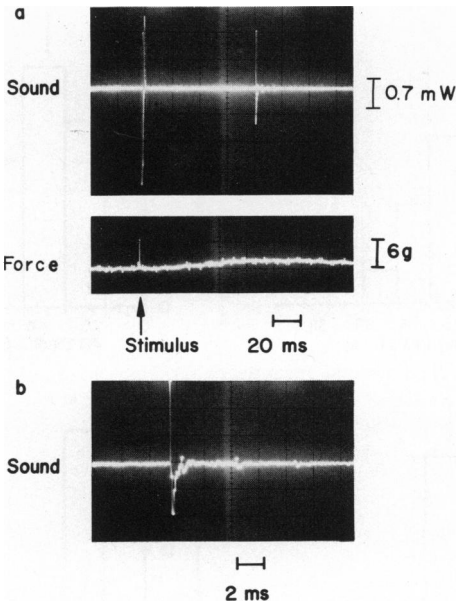

FIGURE 6 The same sound burst is displayed with two different time scales using a digital storage oscilloscope. Stimulus artifact is labeled and in (*a*) the force record is displayed.

the stimulus artifact was measured with the same transducer. This was found to be equal to the duration of the stimulus pulse, nominally 1,000 μs. But, in several experiments, the stimulus duration was varied from 100 to 10,000 μs, as recorded faithfully by the transducer.

In records in which multiple sound bursts were recorded, the interval between successive sound spikes was measured, and the Q_{10} determined two ways. For a group of four muscles at 4.0°C, the median of the interburst interval was 30 ms; when the temperature was raised to 12.4°C the median of this interval, in these muscles, decreased to 18 ms, yielding a Q_{10} of 2.0 (Fig. 7 *a*, *b*). A similar measurement was made for a group of 24 different muscles, each set at one of two different temperatures. For 11 muscles at 3.4°C, the median of the interval between successive bursts was 35 ms and for 13 different muscles at 13.4°C the interval median was 18 ms; giving a Q_{10} of 1.9 (Fig. 7 *c*, *d*).

Stimulation without the muscle in the chamber did not produce sounds; nor did subthreshold stimulation. Also, no sounds were recorded during the period between contractions. When a glass reflector (absorption coefficient = 0.02 at 2 kHz; Kinsler and Frey, 1962) was placed across the full width of the chamber, blocking sound transmission from the contracting muscle to the transducer, only the stimulus artifact was recorded. Sound production returned when the reflector was removed. Movement of the lever arm without the muscle in the bath, produced by manually moving the part of the arm not immersed in the chamber, did not produce sounds.

DISCUSSION

The results of this study confirm in vivo indications that shortening muscles generate sounds. In the majority of

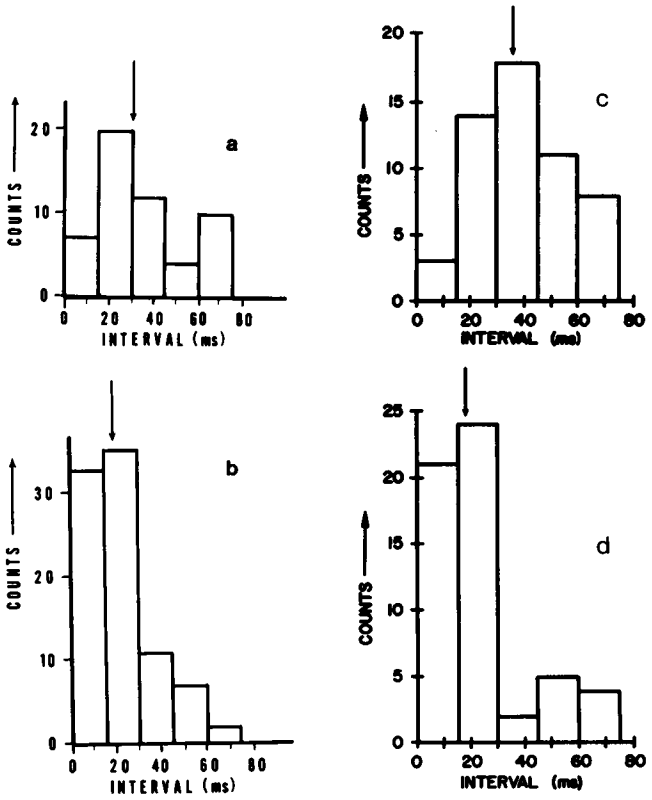

FIGURE 7 Histogram of the interburst interval for 4 muscles at 4.0°C (*a*) and 12.4°C (*b*). (*c*) Histogram of the interval between successive sound bursts for 13 muscles at 3.4°C and (*d*) 11 different muscles at 13.4°C. Medians are indicated by the arrows.

muscles studied, we were able to record sounds of relatively high intensity. The sounds were not continuous; they exhibited a discrete, spikelike character.

Comparison with Previous Studies

In early investigations, contracting muscles were reported to generate continuous, low-frequency sounds. Wollaston (1810) placed a stethoscope on the skin to record the sounds produced by voluntary contraction of the muscles. A low rumbling noise was heard, and its frequency was estimated to be between 20 and 30 Hz. This noise was attributed to vibrations of the muscle fibers. Similar findings were reported by Herroun and Yeo (1885), who used a stethoscope to record a rumbling noise of frequency 36–40 Hz during muscle shortening produced by electrical stimulation or voluntary contraction of the forearm. More recently, Oster and Jaffe (1980) reported that when an electronic stethoscope, which had its output filtered to include only frequencies <60 Hz, was placed on the skin above a muscle that was electrically stimulated or contracting voluntarily, sounds of 25 Hz were recorded.

Recording the sounds produced by large skeletal muscles using a stethoscope, as in the above studies, cannot be expected to yield any more than a continuous low frequency rumble, even if spikes were present, because of the stethoscope's limited bandwidth. Furthermore, such a device averages the activity of many muscle units so that sound spikes generated by individual muscle fibers might be summed and recorded as a continuous low frequency rumble.

On the other hand, our results are comparable to those of Gordon and Holbourn (1948) who reported that during closing of the eyelids, regular discrete sound bursts of 5–15 ms duration were recorded with a small piezoelectric sound detector placed on the skin. The interval between successive sound spikes was proportional to the force of contraction. They attributed the production of sound spikes to pressure waves caused by lateral expansion of the muscle units.

Potential Artifacts

Several potential artifacts that could have produced the sounds were checked. Ambient vibrations were ruled out because sound spikes were not recorded during rest or during subthreshold stimulation. Manually induced movements of the lever in the bath did not yield sound spikes; furthermore, in early experiments where an elastic thread was used instead of the lever, the sounds were similar. Some aberrant electrical signal emerging from the stimulator was deemed unlikely because only the stimulus artifact (before the twitch) remained during either submaximal stimulation, stimulation without the muscle in the chamber, or when an acoustic reflector was placed between the muscle and the sound transducer. Finally, it is possible that the nerve terminals may have fired and produced "sounds," but because the nerve cells had been cut in dissection, it is unlikely that the myoneural junction could have been responsible. This is also supported by the fact that the acoustic reflector, which allowed electrical but not acoustic signals to pass, eliminated the sounds.

Several pieces of evidence favor the muscle origin of the sounds. First, the bursts were moderately repeatable from contraction to contraction (Fig. 4). Second, the interval between successive sound bursts had a Q_{10} of ~2 (Fig. 7), which is similar to the Q_{10} of heat liberation, 2.05 (Hill, 1939). These observations suggest that the generation of sound spikes is a physiological property of the muscle.

Implications

The fact that sounds are generated in discrete bursts and not as a continuous tone indicates that contraction may occur in a discrete, synchronous manner. This is consistent with recent observations that muscle shortening occurs in steps; i.e., periods of high-velocity shortening, which are referred to as steps, alternate with periods of nearly constant sarcomere length, or pauses. Such shortening and lengthening has been observed using several different optical techniques in whole muscles (Pollack, ter Keurs and Iwazumi, unpublished), single skeletal muscle fibers (Pollack et al., 1977, 1979; Delay et al., 1981; Jacobson et

al., 1981 *a*, 1981 *b*) and cardiac muscle (Pollack et al., 1977; Vassallo and Pollack, 1982).

Sound production by a shortening muscle could be explained in the following manner: Because muscle has a constant volume, its width increases during shortening (Elliot et al., 1967; Matsubara and Millman, 1974; Huntsman et al., 1979). A stepwise change in fiber length should thus cause a stepwise change in fiber radius. If enough fibers were to contract in synchrony, this would cause a sudden increase in muscle radius, generating a brief shock wave that should be detectable as a discrete sound burst (Fig. 1).

If the above hypothesis is correct, the period of each sound spike would be indicative of true step duration at the molecular level. It is assumed that each shortening step generates a single sound spike. Thus, the period of the sound spike implies a step duration of 400 μs. This is in the same range as step durations measured using optical techniques (Pollack et al., 1977, 1979; Delay et al., 1981; Jacobson et al., 1981 *b*). In these studies, shortening steps had durations ranging from 200 μs to several milliseconds. The variability may reflect averaging of regions slightly out of synchrony. The data obtained here imply that true step duration at the molecular level may be consistently near 400 μs.

In a whole muscle, sound bursts are probably generated only when several fibers, in a single region or in several regions, shorten synchronously. The intensity of the sound burst would then be reduced by the damping of surrounding fibers and phase interference from sound spikes generated by other fibers which are contracting out of synchrony. The variable intensity of the sound spikes may be due to the variable size of the synchronous region.

In single muscle fibers, steps are repeatable; however, they do not necessarily repeat in every contraction (Pollack et al., 1977, 1979). This observation is consistent with the moderate repeatability of the sound spikes.

The peak acoustic power of 1.1–3.8 mW measured from each burst corresponds to 5–18 mW/g. However, sound spikes have a duration of only 400 μs and occur every 20–40 ms. Therefore, average acoustic power generated during shortening is only 1–2% of the peak value, or between 0.05–0.4 mW/g. This energy is still relatively high; it is on the order of 1% of the 10–20 mW/g of heat produced during a twitch (Hill, 1939).

A theoretical estimate of the acoustic energy generated during shortening correlates reasonably with these measurements. Assume the sartorius muscle is a cylindrical source of constant volume (Fig. 1). Sound would be generated during the radial expansion associated with each shortening step. Acoustic power may be calculated as follows (Hueter and Bolt, 1955):

$$\text{Power} = (v^2 ZS)/2$$

where, v is the rate of change of the radius; Z is the acoustic impedance of the medium; and S is the surface area of the source.

For a cylindrical source 30 mm long and 3 mm in diameter, comparable to dimensions measured in these specimens, the value of S may be computed as 280 mm^2. Z is generally taken as 1,500 g/mm^2 · s (Kinsler and Frey, 1962). Typical values for v may be computed from published records of stepwise shortening (Jacobson et al., 1981 *b*); from Fig. 4 *a* of the latter paper, measured step size is 11 nm/sarcomere ($\simeq$0.5% of muscle length) and step duration is 1 ms. These parameters yield a value of v equal to 3.8 μm/ms. From Fig. 4 *b* of the same paper, v may be calculated as 6.2 μm/ms. The power calculated from these two sets of parameters is 3.1 and 8.2 mW, respectively. If we presume less than total synchrony, these figures would be somewhat lower; perhaps comparable to the 1.1–3.8 mW measured experimentally.

The rough agreement between the theoretical estimates and experimental measurements implies that the sound intensity generated during each burst is indeed on the order of 10 mW/g. Although average acoustic power generated during shortening is much less than this peak value, it is interesting that the peak acoustic power is similar in magnitude to the rate of heat evolution.

Received for publication 30 April 1981 and in revised form 2 September 1982.

REFERENCES

Brozovich, F. V., and G. H. Pollack. 1981. Contracting muscle generates sound bursts. *Biophys. J.* (*Abstr.*). 33:224 *a*.

Delay, M. J., N. Ishide, R. C. Jacobson, G. H. Pollack, and R. Tirosh. 1981. Stepwise sarcomere shortening: analysis by high speed cinemicrography. *Science* (*Wash., D. C.*). 213:1523–1525.

Elliot, G. F., J. Lowy, and B. M. Millman. 1967. Low angle x-ray diffraction studies of living muscle during contraction. *J. Mol. Biol* 25:31–45.

Gordon, G., and A. H. S. Holbourn. 1948. The sounds from single motor units in a contracting muscle. *J. Physiol.* (*Lond.*). 107:456–464.

Herroun, E. F., and G. F. Yeo. 1885. A note on the sound accompanying the single contraction of skeletal muscle. *J. Physiol.* (*Lond.*). 6:287–292.

Hill, A. V. 1939. The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Med.* 126:136–193.

Hueter, T., and R. Bolt. 1955. Sonics. John Wiley & Sons, Inc., New York. 200–256.

Huntsman, L. L., D. S. Joseph, M. Y. Oiye, and G. L. Nichols. 1979. Auxotonic contraction in cardiac muscle cell segments. *Am. J. Physiol.* 237(2):H131–H138.

Jacobson, R. C., R. Tirosh, M. J. Delay, and G. H. Pollack. 1981 *a*. Real time high speed sarcomere length measurements from optical image. *Biophys. J.* 33:223 *a*.

Jacobson, R. C., R. Tirosh, M. J. Delay, and G. H. Pollack. 1981 *b*. Quantized nature of sarcomere shortening steps. *J. Gen. Physiol.* Submitted.

Kinsler, L. E., and A. R. Frey. 1962. Fundamentals of Acoustics. Second Edition. John Wiley & Sons, Inc., New York. 5–252.

Matsubara, I., and B. M. Millman. 1974. X-ray diffraction studies on cardiac muscle. *Ciba Found. Symp.* 24:31–37.

Martin, R. W. 1977. Power transfer effects of backing impedance with thickness mode ultrasonic transducers under complex conjugate matching. *IEEE Ultrason. Symp. Proc.* 416–421.

Martin, R. W., and R. A. Sigelmann. 1975. Force and electrical thevenin equivalent circuits and simulations for thickness mode piezoelectric transducers. *J. Acoust. Soc. Am.* 58:475–489.

Nolle, A. W., and S. C. Mowry. 1948. Measurement of ultrasonic bulk-wave propogation in high polymers. *J. Acoust. Soc. Am.* 20:432–439.

Oppenhein, A. V., and R. W. Schafer. 1975. Digital Signal Processing. Prentice Hall, Inc., Englewood Cliffs, NJ. 5–27.

Oster, G., and J. S. Jaffe. 1980. Low frequency sounds from sustained contraction of human skeletal muscle. *Biophys. J.* 30:119–127.

Pollack, G. H., T. Iwazumi, H. E. D. J. ter Keurs, and E. F. Shibata. 1977. Sarcomere shortening in striated muscle occurs in stepwise fashion. *Nature (Lond.).* 268:757–759.

Pollack, G. H., D. V. Vassallo, R. C. Jacobson, T. Iwazumi, and M. J. Delay. 1979. Discrete nature of sarcomere shortening in striated muscle. *In* Cross-Bridge Mechanism in Muscle Contraction. H. Sugi and G. H. Pollack, editor. University of Tokyo Press, Tokyo. 23–40.

Rooney, J. A. 1973. Determination of acoustic power outputs in the microwatt-milliwatt range. *Ultrasound Med. Biol.* 1:12–16.

Vassallo, D. W., and G. H. Pollack. 1982. The force velocity relation and stepwise shortening in cardiac muscle. *Circ. Res.* 51:37–42.

Wollaston, W. H. 1810. On the duration of muscle action. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 1–5.