EFFECTS OF HIGH INTENSITY SOUND ON ELECTRICAL CONDUCTION IN MUSCLE

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TWELVE FIGURES

INTRODUCTION

In recent years there has been considerable experimentation on the biological and medical applications of ultrasound. Pertinent literature can be located by reference to bibliographies (Naimark et al., '51; Curry et al., '51) and symposium publications (Matthes and Reeh, '49; Giacommi, '50; Fry et al., '53). Some of this research has dealt with effects on muscle tissue (Gersten, '53 and '54; Gary and Gerendas, '49; Harvey, '29). In much of the reported work there is no adequate description of the characteristics of the sound field so that quantitative physical interpretations are difficult.

In this paper, the results of a study of the effect of intense acoustic radiation on the propagation of the action potential in muscle tissue are presented. Temperature measurements in the muscle were accomplished during irradiation, and the effect of pure heating on the electrical conduction process was investigated. The results indicate that irreversible suppression of the propagated action potential by the sound can be accomplished in the absence of a damaging temperature level. Although no experimental work was performed under a hydrostatic pressure sufficiently high to insure that no tension forces existed in the muscle, it is possible to conclude.

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on the basis of indirect observations that no extensive cavitation occurred in the muscle tissue.

EXPERIMENTAL METHODS

1. Ultrasonic generation

A travelling wave sound field, frequency 991 ke/sec., was used for most of the experimental work. This field was pro-

![Graph showing acoustic pressure distribution](image)

*Fig. 1* Acoustic pressure distribution in the sound field. The zero of the coordinate system is the point at which the muscles were centered in the field. The value of the relative pressure amplitude at this point is arbitrarily taken equal to 100.

duced in degassed Ringer's solution by a flat, one inch diameter x-cut quartz crystal mounted as previously described (Fry, '50). The sound field distribution produced by the crystal system and measured at the muscle location is shown graphically in figure 1. The system was calibrated with a thermocouple probe described by Fry and Fry ('54). The intensity and acoustic pressure amplitude, as determined from measurements made with this device at the spatial peak
of the field, are given in table 1 as a function of the voltage applied to the crystal. The biological preparations were subjected to single pulses (square wave envelope) of radiation. The temperature of the solution in which the muscles were immersed could be adjusted to any desired value.

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>CRYSTAL VOLTAGE</th>
<th>PRESSURE AMPLITUDE</th>
<th>INTENSITY</th>
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<tbody>
<tr>
<td>1</td>
<td>8000 volts</td>
<td>19.0 atmospheres</td>
<td>123 watts/cm²</td>
</tr>
<tr>
<td>2</td>
<td>8000 volts</td>
<td>22.2 atmospheres</td>
<td>168 watts/cm²</td>
</tr>
<tr>
<td></td>
<td>7000 volts</td>
<td>16.6 atmospheres</td>
<td>94 watts/cm²</td>
</tr>
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<td></td>
<td>6000 volts</td>
<td>14.2 atmospheres</td>
<td>69 watts/cm²</td>
</tr>
<tr>
<td>2</td>
<td>8000 volts</td>
<td>19.4 atmospheres</td>
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</tr>
<tr>
<td>1</td>
<td>8000 volts</td>
<td>16.6 atmospheres</td>
<td>94 watts/cm²</td>
</tr>
</tbody>
</table>

2. Biological preparation

Excised hind leg biceps muscles from Rana pipiens were utilized in these studies. They were mounted in the holder as shown in figure 2. Irradiation was carried out with the muscle immersed in Ringer's solution. To eliminate cavitation during irradiation, the solution was degassed by boiling for 10 minutes and then cooled rapidly. The preparation was positioned, transverse to the direction of propagation, at the peak of the sound field by means of a probe and pointer system. The positioning procedure was found to give the same location repeatedly within one millimeter. Any nerve or nerve-muscle endplate effects were eliminated by curarizing the preparation to block nerve-muscle stimulation. The muscle fibers were excited directly by a square wave electrical pulse. The curarization was carried out by introducing d-tubocurarine chloride into the Ringer's solution at a concentration of 6 mg per liter. This concentration is about 5 times the dosage found by Kuffler ('42) to be necessary to completely block the nerve-muscle excitation path for single muscle fibers. For complete curarization, checked by electrical
test, the muscle was permitted to remain in the solution for 20 minutes before irradiation.

The experimental procedure followed was to excite the muscle with a pair of electrodes at one end, record the propa-

Fig. 2  Muscle mounted in holder. The electrical leads to both ends are shown.

Fig. 3  Propagated muscle action potential obtained with the arrangement illustrated in figure 2.
gated action potential with a pair of electrodes at the opposite end and irradiate with sound between the pairs of electrodes. A propagated muscle action potential obtained under the experimental conditions outlined is shown in figure 3. No special attempt was made to obtain monophasic responses since this would have complicated the experimental procedure. The amplitude of such an action potential was the quantitative measure used throughout the experiments.

![Graph](image)

Fig. 4 Variation in the amplitude of the action potential of a muscle stimulated once every five seconds.

EXPERIMENTAL RESULTS

1. Effects of acoustic irradiation

Before proceeding to the irradiation studies, electrical control measurements were carried out on a number of muscle preparations. A typical graph of the relative amplitude of the action potential as observed on a muscle stimulated approximately once every 5 seconds for a minute and a half is shown in figure 4. This set of measurements and others
like it demonstrate that deviations from an average value of less than about 5% can be obtained by this technique. The stability of the preparation using the experimental procedure described is therefore quite good.

The most pronounced effect obtained from irradiation was a large permanent suppression, even complete block, of the action potential after exposure to a single pulse of sound.

Fig. 5 Effect of acoustic radiation on the action potential of muscle. The propagated electrical responses to successive stimuli are ordered in columns. See figure 6 for a plot of relative amplitude.
This effect was established by measurements made on about 30 muscles. A typical run of data taken at 20°C, with a peak acoustic pressure amplitude of 19 atmospheres and a 40 second pulse of sound is shown in figure 5. The relative amplitude as a function of time is presented graphically in figure 6. Monotonically decreasing curves have been obtained

![Graph showing relative amplitude of action potential over time](image)

**Fig. 6** Relative amplitude of the action potential of muscle as affected by acoustic irradiation. Values obtained from the records of figure 5. A length of the muscle between stimulating and receiving electrodes was subjected to the sound. Acoustic pressure amplitude, 19 atm.; period of irradiation, 40 sec.; bath temperatures, 20°C.

in addition to the type illustrated which shows a small rise during the period of suppression by the sound. No fundamental significance is attached to this small rise. The muscle was stimulated to twitch about once every 5 seconds. Four control measurements were taken before the sound was turned on and 4 more after the sound was turned off. From the figure and the graph it is apparent that propagation of the action potential was completely blocked by the sound. This effect
is obtainable under various dosage conditions. Figure 7 shows a graph of results using a peak pressure amplitude of 16.6 atmospheres with a 100 second pulse. Similar effects can be obtained at lower temperatures. Figure 8 shows a run taken at 15°C. The amplitude of the action potential is presented graphically in figure 9. In this case about 75% suppression was obtained. A 60 second pulse of sound at 19

![Graph](image)

**Fig. 7** Relative amplitude of the action potential of muscle as affected by acoustic irradiation. A length of the muscle between the stimulating and receiving electrodes was irradiated. Pressure amplitude, 16.6 atm.; period of irradiation, 100 sec.; bath temperature, 20°C.

atmospheres was used in obtaining this result. This run, in conjunction with others made at lower temperatures, indicates that the dosage conditions required to produce the observed effect on muscle are temperature dependent.

In order to derive more quantitative information from the experiments than that provided by a single dosage condition, data were obtained for a curve of minimum dosage required to produce a prescribed change in the electrical conduction
Fig. 8 Effect of acoustic radiation on the action potential of muscle. The propagated electrical responses to successive stimuli are ordered in columns.
characteristic of the biceps muscle. Minimum dosage was defined in the following manner. For each irradiation time, three muscles were subjected to the sound at each of several driving voltages across the crystal. These were spaced 250 volts apart, this increment representing less than 5% of the total driving voltage. The pressure amplitude of minimum dosage for a specific duration of irradiation was defined to lie between the pressure amplitude corresponding to the voltage at which at least two of the three muscles experienced a reduction of more than 10% in action potential amplitude and that pressure amplitude at which no more than one muscle was so affected. A further proviso was required that at higher pressure amplitudes than the first, a greater reduction in action potential must be observed, and at pres-
sure amplitudes below the second, the observed effects must be smaller. The reciprocal of the irradiation time was plotted against the sound pressure amplitude of minimum dosage to yield the curve of figure 10. The temperature range over which all these data were obtained was between 18 and 20°C.

Fig. 10 Reciprocal of the minimum duration of irradiation to obtain a permanent reduction of the muscle action potential as a function of the acoustic pressure amplitude. See text for a precise definition of minimum dosage.

Over 120 frogs were used in obtaining the measurements on minimum dosage. The straight line relationship of the data is similar to the curve obtained by Fry (’51) from data on the paralysis of frogs irradiated in the lumbar enlargement region of the spinal cord. It is of interest to note here that the induction by ultrasound (lower in pressure amplitude than the levels used in the experiments reported in this paper)
of reversible changes in muscle which can be ascribed to a non-temperature mechanism have been previously reported (Busnel et al., ’53).

It was observed in the course of the experimentation that no difference in effects occurred by switching the muscle end for end. The possible dependence of the observed effects on the geometrical orientation of the muscle with respect to the direction of propagation of the sound was considered. Consequently, experiments were performed at two of the pulse durations used in obtaining the data for figure 10 with the muscle oriented at 60 degrees with respect to the direction of propagation rather than perpendicular to it. These results, plotted in figure 10, indicate that there is no major difference for the orientations tested.

Histological studies were carried out on irradiated muscles stained with hematoxylin-eosin. The tissue was fixed in formalin immediately after irradiation. No differences were observed microscopically between the irradiated and normal tissue. Examination of sections of irradiated tissue indicate no tissue tearing or vacuolization.

Preliminary work on the acoustic irradiation of striated muscle with the vascular system intact (gastrocnemius muscle of Hyla) indicates that results similar to those described in this paper are obtained.

Irradiation of excised sciatic nerve of Rana pipiens at the highest sound level used in the studies reported herein, exposed for much longer periods of time than that required to permanently block muscle action potentials, produces no observable suppression in the nerve action potential.

2. Temperature changes in muscle produced by irradiation

Temperature changes were measured in muscles during irradiation by means of imbedded thermocouples. These thermocouples were made of three mil copper and constantan wires joined with a soldered lap junction. The thermocouple was threaded through the excised mounted muscle. Equi-
librium temperature measurements were made. Practical equilibrium was established in less than 10 seconds after the sound was turned on. The thermocouple was placed in the muscle as close to the center line as possible. As indicated previously the muscle was located at the peak of the sound field by means of a probe and pointer. The thermocouple emf was observed for various sound levels so that a curve could be obtained of temperature change versus acoustic intensity. The thermocouple was calibrated for each muscle while imbedded in the muscle. The holder with muscle and imbedded thermocouple was placed in baths of Ringer's solution at various temperatures and emf readings were taken. The temperatures of the baths were measured with a mercury thermometer and a calibration curve of thermocouple emf as a function of temperature was plotted.

Fig. 11 Equilibrium temperature rise in a muscle at various sound levels determined by measuring the emf generated by an imbedded thermocouple.
Data of the type described were taken on 10 muscles. A typical thermocouple calibration curve and temperature measurement curve for a muscle are shown in figure 11. In all but one of these instances, a plot of temperature rise as a function of the square of the crystal voltage demonstrated a linear relationship. The one set of non-linear data was therefore disregarded as representing an atypical situation, possibly caused by cavitation at the thermocouple-muscle interface. Table 2 shows values of the equilibrium temperature rises in muscles for peak sound intensities of 123 and 94 watts/cm². At the higher acoustic level it is possible to block completely

<table>
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<tr>
<th>MUSCLE NUMBER</th>
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<th>TEMPERATURE CHANGE AT 123 WATTS/CM²</th>
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<td></td>
<td>°C.</td>
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</tr>
<tr>
<td>9</td>
<td>9.6</td>
<td>12.5</td>
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the muscle response in about 40 seconds. At the lower level about 100 seconds are required. It is apparent from table 2 that quite a wide range of values for the equilibrium temperature rise is obtained in various muscles. The reasons for this are readily understood on the basis of the following discussion.

In the problem being considered, the muscle is roughly the shape of a long cylinder and is imbedded in a large bath of Ringer’s solution which is maintained at essentially constant temperature. Transverse to the axis of the muscle, the sound intensity varies less than 10% over the muscle. In the axial direction the beam is about 7 mm wide at the half power points. The muscle is generally quite a bit longer than this.
Two simple geometries for the mathematical solution of the problem suggest themselves for the physical conditions. One is an infinitely long, solid, circular cylinder with a constant amount of heat per unit volume generated throughout and with the surface kept at a constant temperature. Since the sound beam is finite and small, the other suggested geometry is a solid sphere with a constant amount of heat per unit volume generated throughout and with the surface kept at a constant temperature. The cylindrical model leads to predicted temperature values in the muscle which are high, since heat is not actually supplied all along the cylinder but only in a small region. The second geometry probably leads to low values of predicted temperature rise, since part of the boundary is then not Ringer’s solution but is muscle and does not remain at the fixed temperature of the Ringer’s solution.

For the cylindrical situation, under equilibrium conditions, the solution to the general heat flow equation is (see for example Carslaw and Jaeger, ’47)

\[ T - T_i = \frac{A(a^2 - r^2)}{4k} \]  \hspace{1cm} (1)

while for the spherical case

\[ T - T_i = \frac{A(a^2 - r^2)}{6k} \]  \hspace{1cm} (2)

In equations (1) and (2), \( T_i \) is the constant temperature of the Ringer’s solution and \( T \) designates the temperature in the muscle. The symbol \( r \) represents the radial coordinate and \( a \) is the radius of the muscle. The symbol \( k \) designates the heat conductivity coefficient of the muscle and \( A \) is the heat generated per second per unit volume resulting from absorption of acoustic energy. These equations demonstrate that the solutions for the temperature distribution as a function of radial coordinate are identical in form and differ for any value of \( r \) by only 33% in magnitude. Since these are extreme situations with the physical case falling somewhere between, it seems reasonable to use these solutions to describe approximately the actual condition in the muscle. Comparison of the temperature changes predicted by the equations
with the measured values can be made by inserting appropriate values of the constants into equations (1) and (2).

The value of $A$, the heat generated per unit volume per second, is given by

$$A = I \mu$$

(3)

where $I$ designates the sound intensity and $\mu$ the intensity absorption coefficient per unit path length. In order to calculate values corresponding to the measured results given in table 2, intensities of 123 and 94 watts/cm$^2$ were used. The value of $\mu$ for striated frog muscle was found by Fry et al. ('50) to be about 0.2. For lack of more complete information, the value of the thermal conductivity coefficient of water ($k = 0.0060$ watts/cm$^2$/°C. at 20°C.) was used in

<table>
<thead>
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<th>Table 3</th>
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<td>Computed temperature changes along the axis of a model of a muscle under sound irradiation.</td>
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</table>

<table>
<thead>
<tr>
<th>SOUND INTENSITY (watts/cm$^2$)</th>
<th>TEMPERATURE RISE (°C.) CYLINDRICAL GEOMETRY</th>
<th>TEMPERATURE RISE (°C.) SPHERICAL GEOMETRY</th>
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</thead>
<tbody>
<tr>
<td>133</td>
<td>16.3</td>
<td>6.9</td>
</tr>
<tr>
<td>94</td>
<td>7.9</td>
<td>5.3</td>
</tr>
</tbody>
</table>

the calculations. Measurements of the diameters of 50 muscles indicated an average value of $a = 0.1$ cm. Table 3 shows the computed values of peak temperature rise ($r = 0$) for the two geometries at the two sound intensities chosen.

A comparison of tables 2 and 3 demonstrates that the agreement between calculation and measurement is good. An examination of equations (1) and (2) helps explain the variation in the measured values. Part of the variation may be accounted for by inaccurate positioning of the thermocouple. For example, if the thermocouple is positioned at half the radial distance away from the axis of the muscle, then the temperature rise will be only three quarters of the peak value ($r = 0$). Much of the variation can be accounted for by differences in muscle size. Since the equilibrium temperature rise is proportional to the square of the muscle radius,
an increase in this value of only 25% leads to a change in the value of the temperature rise of more than 50%.

A pertinent point to note from the equations is that the temperature rise at any radial distance is proportional to the quantity \( (a^2-r^2) \), therefore half the muscle fibers undergo a temperature rise of less than half the peak value.

Fig. 12. Relative amplitude and duration of the action potential of frog biceps muscle as a function of the temperature.

3. Effects of temperature changes on muscle action potential

Study of some of the literature in the field (Doi, '20; Walker, '49) yielded little conclusive information on the effects of temperature changes on the action potential of muscle. Experiments were therefore undertaken to determine the effects of temperature changes on the frog muscle action potential under conditions similar to those established for the acoustic irradiation experiments. Measurements were carried out on 7 muscles under the following conditions. A number of saline-
tubocurarine baths were kept at various fixed temperatures and each muscle was run through the different baths. The muscle was kept in each bath for two minutes to bring about equilibrium conditions before electrical measurements were made. This procedure yielded consistent results. In 6 of the 7 muscles, almost complete reversibility in height and duration of the action potential was measured as the muscle was carried through a temperature cycle from about 20°C. to about 35°C. then back to 20°C. In no case was the amplitude of the action potential reduced to zero. A graph of the relative changes in amplitude and duration of the action potential as a function of temperature is shown in figure 12 for a typical muscle. These measurements of the effects of temperature change on the action potential demonstrate that if acoustic absorption in the muscle does not result in a temperature greater than 35°C. throughout the muscle, permanent reduction or irreversible block of the action potential cannot be attributed to the temperature change.

DISCUSSION OF RESULTS

Before considering the physical mechanism of the action of sound on muscle tissue, it is necessary to eliminate possible artifacts. Hydrodynamical flow, which is present in intense sound fields in fluids, might cause mechanical difficulties at the connection of the electrodes to the muscle. In addition, direct mechanical forces on the electrodes, such as radiation pressure, might contribute to the observed effects.

In investigating the hydrodynamic flow artifact possibility, experiments were carried out with flows produced by ejecting fluid from a jet immersed in the coupling liquid and directing it at the muscle. The effects on the observed action potential, caused by flow velocities 6 to 10 times those present during acoustic irradiation, are slight and indicate that the results obtained during ultrasonic irradiation (large reduction or complete block of the action potential) are not caused by a flow artifact.
Two types of experiments were carried out to eliminate the possibility of direct electrode artifacts. When a balsa wood fixture was mounted on the muscle holder for the purpose of shielding the electrodes from the sound field, large reductions of the height of the action potential were still attainable under ultrasonic irradiation. When a pair of electrodes was placed at the peak of the acoustic field and irradiated with a sound level equivalent to that normally incident on the electrodes for the customary geometry only slight changes in the observed response were evident. Therefore electrode artifacts do not account for the observed ultrasonic effects.

Besides these artifacts, it is always possible that the observed effects can be caused by heating or cavitation in the muscle tissue. Finally, the mechanical forces which are inherent in a sound field travelling through a medium can be involved in the mechanism. These forces can be unidirectional, such as radiation pressure or Oseen type, or sinusoidally varying with time such as the oscillatory viscous force associated with the relative motion between a fluid medium and imbedded particles.

The possibility that the observed irreversible suppression of the action potential is caused by excessive muscle heating has been eliminated. An examination of the irradiation data indicates that it is possible to block permanently or greatly reduce the amplitude of the action potential with the muscles at initial temperatures of 20°C and 15°C. by irradiating at a peak sound pressure amplitude of 19 atmospheres (123 watts/cm²). The measured values of the temperature rise in the muscle under the same irradiation conditions show that the temperature in the tissue does not exceed approximately 30°C, for an initial temperature of 20°C., and for an initial temperature of 15°C. the maximum temperature does not exceed 25°C. An examination of the effects on the muscle action potential of a temperature cycle reaching a maximum value of 35°C. proves that blocking does not occur and that the amplitude changes are essentially reversible. The experimentally measured values of the temperature rise in the muscle
during irradiation are in agreement with calculated values, and the analysis further indicates that half the fibers in the muscle undergo temperature changes of less than half the maximum value.

In discussing the possible role of cavitation in the mechanism of the observed effects produced in muscle, it is noted that as yet no direct attempt has been made to completely eliminate the cavitation possibility by carrying out the experiments under a hydrostatic pressure sufficiently high to insure that no tension forces exist in the tissue. Such experiments are currently in the planning stage. However, there are a number of indications that cavitation was not present, at least to any appreciable extent, under the experimental conditions which prevailed. The first evidence comes from an examination of the thermocouple measurements of temperature rise in the muscle under sonic irradiation. These measurements indicate that for peak sound pressure amplitudes up to about 20 atmospheres the temperature change is proportional to the square of the crystal voltage. Marked deviation from this linear relationship would be expected in the presence of cavitation. Many investigators have demonstrated that sound level measurements, in a medium in which cavitation is present, become erratic. A second piece of evidence against excessive cavitation in the tissue under irradiation results from an examination of the stained sections of irradiated muscle. Microscopic study shows no vacuole formation or fiber tearing. Another argument against the possibility that cavitation is involved in the mechanism of the effect produced in these experiments is brought out by the work of Esche (52) in which he found no evidence of cavitation at a hydrostatic pressure of one atmosphere and a frequency of 500 kc/sec, in beef muscle up to sound intensities in the tissue of about 400 watts/cm².

It appears that neither tissue heating nor cavitation is the primary physical factor involved in producing irreversible block of the action potential in striated muscle under the experimental conditions described in this paper. The effect of
the high intensity acoustic irradiation of the muscle may therefore be a result of mechanical forces acting on submicroscopic elements of the tissue. From the experimentally observed form of the minimum dosage curve, it is reasonable to expect the mechanism to take a mathematical form approximating that proposed by Fry et al. (51). If the threshold value is chosen as unity for the scale along the horizontal axis for the muscle dosage curve and for the frog paralysis dosage relation (Fry et al., '51) then the two relations appear almost identical. This suggests that the mechanism may well be the same in the two cases. Knowledge of the apparent lack of a dependence of the effect on the angle between the direction of the muscle fibers and the direction of propagation of the sound, should be useful in any attempt to establish a mechanical force mechanism.

SUMMARY

The studies presented in this paper demonstrate that under appropriate ultrasonic dosage conditions, the propagated action potential of an excised striated muscle can be permanently reduced or completely blocked. This can be accomplished in the absence of a temperature level sufficient in itself to cause permanent suppression. A quantitative determination of the minimum dosage relation (duration of exposure as a function of sound level) for a 10% permanent reduction of the muscle action potential has been accomplished. The form of this relation is the same as that given by Fry et al. ('51) for the relation between the minimum exposure time for paralysis of the hind legs of frogs, irradiated in the lumbar enlargement region of the spinal cord, and the sound level. Histological examination of stained tissue sections shows no gross tearing or vacuolization which might be expected if cavitation were present. Measurements made during irradiation with an acoustic probe imbedded within the muscle provide supporting evidence that cavitation is absent.

Results similar to those obtained on excised muscles were obtained on muscles with intact vascular systems.
No permanent suppression of the nerve action potential was produced on excised sciatic nerves under irradiation conditions similar to those used on excised muscles.

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LITERATURE CITED


EFFECTS OF SOUND ON MUSCLE


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