EFFECTS OF HIGH INTENSITY SOUND ON ELECTRICAL CONDUCTION IN MUSCLE

BY

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THESIS

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______________

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CHAPTER I

INTRODUCTION

This investigation of the effects of high intensity sound on muscle tissue was undertaken as part of a general study of the physical effects of sound on vertebrate tissue. The philosophy behind the undertaking was that the advantages of sound as a biological research tool and as a medical instrument could only be realized by a complete understanding of the physical phenomena that are involved. This understanding should include a physico-mathematical mechanism that will explain many of the mechanical effects on the diverse biological structures, for it is felt that only in this fashion can great advances be made in the field.

Sound has been of interest to the medical field for some time both in its constructive and destructive aspects. In the past eight years it has been used as a clinical instrument in the treatment of a large number of diseases. For many more years there has been the problem of coping with the bodily malfunctions that arise from the use of some vibrating industrial tools such as air hammers. With recent industrial developments this latter problem may be accentuated. While in most cases it is not definitely known, for both the destructive and constructive aspects of the sound, what tissues are being affected, it is anticipated that the damage dosage level studies carried out in the course of this investigation will be of use in determining whether muscle tissue could be the sensitive element.

28. Superscripts refer to numbers in the Bibliography.
It is possible that as a biological research tool sound can throw some light on the problems of structure of nerves and muscles. This is most probable under experimental conditions so controlled that temperature and cavitation effects are minimized and therefore direct mechanical effects of the sound wave are observed. For if sound wave forces can break structural elements, then precise observations of minimum dosage required to produce effects should yield quantitative information on structural constants.

Since this investigation deals with muscle, and in particular striated skeletal muscle, some understanding of the general anatomical and physiological properties of muscle is necessary for an evaluation of experiments of the nature to be described. Muscles perform many of the mechanical activities of the body such as those concerned with locomotion, digestion, excretion, and reproduction. This is in contrast to the nerves which perform many of the information bearing functions. The muscles can generally be divided into two groups. There are those muscles which are attached to skeletal members and are muscles of movement. Examples of such muscles are leg or arm muscles. Other muscles are arranged around sacs of fluid and are generally holding muscles. Stomach and intestines are examples of such muscles. Microscopically these two types of muscle fall into structural classifications of striated or smooth respectively. Cross striated muscle is made up of alternating regions which are optically isotropic and anisotropic. Thus under illumination as viewed through a microscope, the muscle fibers appear to be striped. Smooth muscle fibers in general do not have this optical property. All the muscle studies to be described were carried out on striated skeletal muscles and most of the discussion throughout this work will apply to only those muscles.
The mechanical contraction of muscle fibers is triggered by an electrical pulse from a nerve fiber connected to the skeletal muscle fiber by an endplate which carries out the required electrochemical activity. A single nerve pulse gives rise to a propagated all or none contractile response, or twitch, in a single muscle fiber. Along with this mechanical wave which is propagated along the muscle fiber (at about 1-2 m/s for frog skeletal muscle) there is a measurable propagated electrical pulse, or action potential. Some theories indicate that this action potential acts as the continual trigger for the mechanical wave as the electrical pulse propagates along the fiber membrane. In any event, in twitch type contractions the electrical and mechanical pulses appear always to go hand in hand. It is apparent that this action potential provides an excellent means of electrically measuring and displaying the contracting activity of a muscle. An example of a propagated muscle action potential as measured with two electrodes on a whole muscle (or bundle of fibers) is shown in Fig. 1. While the discussion so far has indicated that a muscle fiber is triggered by a nerve impulse, it is possible chemically to block this nerve trigger action, and furthermore it is possible to excite a muscle fiber directly by applying an electrical pulse of sufficient amplitude to electrodes attached to the muscle. In this manner one can separate the effects of the experimental environment upon the muscle from effects upon the nerve or nerve-muscle endplate. The drug, curare, is satisfactory for blocking the nerve impulse triggering action since it apparently blocks the activity at the endplate without otherwise harming the preparation. The experiments on frog muscles that are described in this report were all carried out on curarized preparations with the muscle directly excited by an electrical pulse.
Although a tremendous amount of experimentation has been carried out on biological and medical applications of ultrasound\textsuperscript{5,25} it is usually quite difficult to make comparisons between the various studies, since the physical constants of the experiments (sound level, frequency, and spatial distribution) are usually not clearly stated, nor perhaps even clearly established, in discussing the problems. In those cases where the sound intensity is given, it is not always clear how it was measured and therefore, whether it is a spatially peak value or an average value measured over a large area. These differences will naturally influence the interpretation of the experimental results.

Figure 1. Muscle Action Potential
With these considerations in mind, reports of some of the sonic irradiations of muscle previously carried out can be presented to give some picture of the results obtainable by the process. Harvey\textsuperscript{21} irradiated heart muscle, smooth muscle, and some striated muscle (gastrocnemius) and while he found some changes in rhythm of the heart beat under irradiation, he found no effects on irradiated curarized gastrocnemius under his experimental conditions. Garay and Gerendas\textsuperscript{17} used a 300 kC sound source which produced a sound beam with an intensity of 10 watts/cm\textsuperscript{2} and after 10 minutes of irradiation they found fibrillar separation in untreated skeletal muscle. They also irradiated muscle preparations from which the actomyosin had been removed and found transverse splitting of the fibers. Szent-Gyorgyi\textsuperscript{26} indicates that actin, one of the contractile proteins in muscle, can be depolymerized by ultrasonic irradiation. Guy Busnel and coworkers\textsuperscript{20} found some changes in chronaxie of muscle under irradiation with a beam of average intensity of 14.5 watts/cm\textsuperscript{2} as measured calorimetrically. Fischer\textsuperscript{9} found on using weak, one megacycle ultrasonic irradiation on normal and denervated skeletal muscle that there was mild extracellular edema 18 hours after irradiation. Herrick\textsuperscript{22} found no evidence of change in superficial muscle of a young rat after exposure to ultrasonic energy, although the nerve fibers exhibited myelin degeneration. Gersten\textsuperscript{18,19} has carried out a number of studies of the effects of ultrasonic irradiation on isometric tension, injury potential and the contractile proteins on frog gastrocnemius. He used average sound intensities up to 3 watts/cm\textsuperscript{2} and found that at this level there appeared to be effects not completely explainable by tissue heating.
None of these studies demonstrated effects similar to the ones considered in this report and comparison is therefore difficult, but this summary does indicate some of the effects that can be obtained with sound on the biology of muscle tissue.

Since it is felt that the maximum information concerning effects of sound on biological tissue can be obtained from experiments which demonstrate direct mechanical action of the sound on tissue, the studies carried out were directed along these lines. In this report it is, therefore, attempted to show that the observed effects were not due to temperature changes or cavitation in the tissue. Many effects of sound on biological systems are caused by heating or cavitation, and in fact one sometimes gets the impression that most effects observed are due to these two causes. It is therefore hoped that this study will demonstrate that direct mechanical effects are possible to achieve.
CHAPTER II

MECHANICAL AND ELECTRICAL EQUIPMENT

In utilizing ultrasonics in biological investigations, experience has indicated that the proper design and use of equipment for the research is perhaps the most important factor in obtaining worthwhile results. The success of this investigation was greatly dependent upon the realization of a system which produced a peak sound intensity greater than 100 watts/cm$^2$ and allowed for repeated positioning of samples to within a fraction of a millimeter of the peak of the sound field. Quantitative measurements on the biological specimen necessitated electronic instruments with accurately controlled properties and with a display of output information in a form which could be readily recorded in a quantitative manner. A block diagram of the system is shown in Fig. 2.

The mechanical equipment was designed for experimentation in which the biological specimen could readily be moved in the sound field projected into the coupling liquid. The crystal projectors used were basically of a type described by F. J. Fry. A number of focussing arrangements were tried out in conjunction with the basic crystal system. These involved cylindrical lenses, spherical lenses, and diffraction barriers; but after examining the beam patterns of the radiating sound field they were all discarded in favor of a flat one inch, one megacycle quartz crystal. This transducer produced a beam pattern that was of a spatial configuration suitable for the experiments and that was stable with time. In the course of the experimental work two such crystal systems were used, one
BLOCK DIAGRAM OF THE APPARATUS
being an improvement over the other in the type of electrical connections being made to the crystal.

The ground plate of the crystal system which faces into the irradiation chamber is fabricated of stainless steel. The irradiation chamber is also of stainless steel and is formed in the shape of an open cylinder with the open side up. This shape reduces the number of welds needed. Stainless steel was used to prevent electro-chemical reaction of the system with the Hinger's solution used to bathe the muscle and acoustically couple the crystal to the muscle. A thin sheet (1/32") of sound rubber (O-c rubber) is used between the irradiation chamber and the sound absorber. A sound absorber was used in the experiments to keep the sound field essentially a travelling wave field. The sound absorber used in these experiments is a brass cylinder ten inches long filled with castor oil. The irradiation chamber and the sound absorber are surrounded by a cooling chamber so that a continuously flowing cold water bath cools the equipment. The coordinate positioning device used has been described by W. J. Fry.12 This device permits continuously variable positioning in three orthogonal directions with a placement accuracy of about $\frac{1}{2}$ millimeter in each direction. The positioning device is securely fastened to the irradiation chamber and crystal projector system so that position settings are accurate with respect to the quartz crystal.

Two types of specimen holders were used for the experiments. The type shown in Fig. 3 was used to irradiate excised muscles. The wires used to make electrical contact to the muscle are 3 mil enamelled silver wires with the enamel cleared from the wires in the region of contact. An enlarged diagram of a wire threaded
**Figure 3.** Excised Muscle Holder

**Figure 4.**

ELECTRODES THREADED THROUGH MUSCLE
through a muscle is shown in Fig. 4. A holder designed for irradiation of muscle with the frog partially or completely intact is shown in Fig. 5. The same type of wires are used to make electrical contact to the muscles in this holder.

Figure 5. Frog Holder

Three types of acoustic probes were used for calibration, plotting of the sound field, and routine checks of the sound level during the period of experimentation. For accurate calibration, a thermocouple probe of the type described by Fry and Fry was used. For the more routine measurements, piezoelectric probes of a type previously described were employed. One of these contains a small piece of X-cut quartz, 2mm by 5 mm in cross-section, as the
transducer element; while the other contains a small section of a barium titanate cylinder, 1 mm by 2.5 mm, as the element. The smaller barium titanate probe was used for field plotting.

Two independent electronic systems were needed. One was used to drive the transducer and the other was used to stimulate and record from the muscle. A block diagram of the driver system is shown in Fig. 6. The basic frequency control in this driver is provided by a Ferris signal generator, Model 22-A. The frequency of the signal generator is monitored by a Signal Corps model BC-221 frequency meter. The signal generator drives a class C amplifier which in turn drives the crystal. The amplifier is capable of supplying slightly more than 8000 volts RMS to a one inch, one megacycle quartz crystal. The voltage across the crystal is measured with a Hewlitt Packard 410 A vacuum tube voltmeter used in conjunction with a capacitive voltage divider. Two types of keying are available between the signal generator and the amplifier. For relatively long irradiation pulses, a mechanical relay controlled by a clock can be used. For the purposes of calibration, when accurately controlled one second pulses of sound are needed, an electronic keyer timed by a 1000 cycle tuning fork can be switched into the circuit.

A block diagram of the stimulating and recording system is shown in Fig. 7. The electronic stimulator is a standard type of square wave generator with an output of about 80 volts on open circuit, a pulse duration variable from 30 microseconds to 300 milliseconds, and a repetition rate variable from about 0.1 c.p.s. to 3000 c.p.s. A variable delay can be inserted into the stimulator circuit for appropriate pictorial display of the results. A General
Ferris Signal Generator
BC 221 Frequency Meter
Clock Controlled Mechanical Relay
Tuning Fork Controlled Electronic Keyer
Class C Amplifier
Power Supply
Vacuum Tube Voltmeter
Sound Projector

BLOCK DIAGRAM OF ELECTRONIC DRIVER
BLOCK DIAGRAM OF ELECTRONIC STIMULATING & RECORDING SYSTEM
Radio 578 A transformer is used to isolate the stimulator from the muscle. An Offner type 140 A amplifier is used in the pick-up system and its output is displayed on a DuMont type 303-A oscilloscope. Permanent records are obtained with a 35 mm oscilloscope camera.

The system as a whole was found to operate quite satisfactorily in the experiments on both excised muscles and on muscles in small frogs.
CHAPTER III

EFFECTS OF SOUND ON THE ELECTRICAL CONDUCTION OF MUSCLE

The effects attainable by irradiating tissue with sound are so highly dependent upon the field configuration and the intensity, that it is appropriate to begin this section with a discussion of the sound fields. Two sound systems were used for all the experiments, and detailed measurements were made on these systems. Figure 8 shows relative field plots in three orthogonal directions for system one. It is apparent that this system produces a sound beam which, at the distance it is being used, is about 7 mm wide at the half power point and is flat over the muscle dimension within a few percent in the longitudinal direction. Much of the variation shown in the longitudinal direction is due to reflections from the face of the probe used to plot the field pattern. The barium titanate piezoelectric probe was used for field plotting. Absolute values of the sound intensity at the spatial peak of the field were obtained with a thermocouple probe\(^\text{15}\) which had previously been calibrated. At 20° C this probe had a calibration of 14.7 watts/cm\(^2\) per centimeter deflection on the galvanometer of the Hathaway oscillograph used in conjunction with the probe, for a one second sound pulse. A plot of galvanometer deflection for such a sound pulse as a function of crystal voltage as measured at the spatial peak of system one is shown in Fig. 9. The frequency of operation for maximum output for crystal system one is about 966 KC. The field patterns for crystal system two are shown in Fig. 10 and the calibration curve is shown in Fig. 11. The beam width of this
CRYSTAL SYSTEM NO. 1 - T = 20.5°C. F = 966.5 KC.
CRYSTAL SYSTEM NO. 1 - CALIBRATION CURVE  T = 20.9°C. - CALIBRATOR
SENSITIVITY = 14.7 W/cm²/cm. (For a one second sound pulse)
Figure 10

Relative Probe Voltage

Distance - Millimeters

CRYSTAL SYSTEM NO. 2 - T = 188°C. F = 990.8 KC
Figure 11

CRYSTAL SYSTEM NO. 2 - CALIBRATION CURVE  \( T = 19.5^\circ \text{C} \) - CALIBRATOR SENSITIVITY = 14.7 W/cm\(^2\)/cm. (For a one-second sound pulse)
second system is about the same as that of system one but the output intensity is about 22 percent greater. Intensities and sound pressure amplitudes for both systems at various crystal voltages that were used are shown in Table 1. These values are for the spatial peak of the field.

**TABLE 1: CALIBRATION DATA FOR THE CRYSTAL SYSTEMS**

<table>
<thead>
<tr>
<th>Voltage Volts</th>
<th>Pressure Amplitude Atmospheres</th>
<th>Intensity Watts/cm²</th>
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</thead>
<tbody>
<tr>
<td>System 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8000</td>
<td>19.0</td>
<td>123</td>
</tr>
<tr>
<td>7000</td>
<td>16.6</td>
<td>94</td>
</tr>
<tr>
<td>6000</td>
<td>14.2</td>
<td>69</td>
</tr>
<tr>
<td>System 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8000</td>
<td>22.2</td>
<td>168</td>
</tr>
<tr>
<td>7000</td>
<td>19.4</td>
<td>128</td>
</tr>
<tr>
<td>6000</td>
<td>16.6</td>
<td>94</td>
</tr>
</tbody>
</table>

Most of the experiments described in this section were carried out on the excised biceps muscle of the hind leg of the frog (Rana Pipiens). The preparation and experimental procedure were of such a nature as to eliminate any nerve or nerve-muscle end-plate effects, and to allow a travelling wave of sound to propagate unimpeded through the muscle. The excised muscle was mounted in its holder as previously described, and then immersed in the Ringer's solution in the irradiation chamber. The sodium bicarbonate was eliminated from the formula in mixing the solution as it was found that bicarbonate tended to make the mixture gassy under sound irradiation. To eliminate cavitation under irradiation, the Ringer's solution was degassed by boiling for ten minutes and then cooling rapidly. The preparation was properly positioned in the sound field in the following manner. The peak of the field was located and fixed with the barium titanate piezoelectric probe. A
pointer was then fixed at the location of the probe and the probe was removed. The center of the muscle was then moved to the tip of the pointer and the pointer was removed. The procedure was found to give the same position repeatedly within about one millimeter. Since this was so, for repetitive runs, the scale readings on the coordinate system were used for positioning successive muscles.

Any nerve effects that might occur were eliminated by curarization. After electrical excitation and pickup were established, d-tubocurarine chloride was introduced into the Ringer's solution at a concentration of 6 mg. per liter. This concentration is about five times the dosage found by Kuffler to be necessary to completely block the nerve-muscle excitation path for single muscle fibers. For complete curarization, the muscle was permitted to remain in the solution for 20 minutes before running tests. It was observed that if the excitation had been taking place at the end of the muscle where the nerve-muscle end plates are located, curarization increased the threshold for excitation quite a bit, while if excitation had been taking place at the opposite end of the muscle, not much change in stimulation level was required to excite the muscle after curarization. A check on the curarization procedure was carried out by injecting approximately 0.3 mg. of d-tubocurarine into one leg of the whole frog, waiting until curarization was complete as demonstrated by the lack of movement of the frog, excising the biceps muscle of the other leg and running electrical tests on it. It was found that the electrical activity after curarization in this manner was the same as that after curarizing by diffusion into the excised preparation.
The experimental procedure used was to excite the muscle into contraction with a pair of electrodes at one end, record the propagated action potential with a pair of electrodes at the other end of the muscle and to irradiate with sound between the pairs of electrodes. Control runs on muscle preparations indicate that the stability of the experimental procedure is quite good. Figure 12 shows pictures taken on a muscle stimulated approximately every five seconds for a minute and a half. A graph of the relative amplitudes of the action potential as taken from the pictures (see Fig. 13) shows that deviations from an average value of less than five percent can be obtained by this technique. The height of the action potential was the quantitative measure used throughout the experiments.

Large permanent suppression and complete block of the action potential after exposure to a single pulse of sound were the most pronounced effects obtained. Such effects were measured on about 30 muscles. A typical run of data taken at 20°C with a peak sound pressure amplitude of 19 atmospheres and a 40 second pulse of sound, is shown pictorially in Figure 14 and graphically in Figure 15. The muscle was stimulated to twitch about every five seconds. Four control pictures were taken before the sound was turned on and four more pictures were taken after the sound was turned off. As is apparent from the graph and pictures, the propagation of the action potential was completely blocked by the sound. These effects are obtainable at different dosage conditions. This is demonstrated in Figures 16 and 17 which show results using a peak pressure amplitude of 16.6 atmospheres with a 100 second pulse of sound. Within certain limits, similar effects can be obtained at lower temperatures. Figures 18 and 19 show a run taken at 15°C. In this case
Figure 12
MUSCLE IRRADIATION STUDY

T = 20°C

SOUND PULSE

19 ATMOSPHERES PEAK

40 SECONDS

Figure 14
Figure 15

IRRADIATION STUDY - Time between stimulation 5.8 seconds - Temp. 20° C.
Sound Pulse - 19 Atmospheres peak Amplitude, 40 seconds.
MUSCLE IRRADIATION STUDY  \( T = 22.5^\circ C \)
SOUND PULSE - 16.6 ATOMSPHERES PEAK, 100 SECONDS

Figure 16
Figure 17

IRRADIATION STUDY - Time between stimulation 7.2 seconds - Temp. 22.5°C
Sound Pulse 16.6 Atmospheres peak amplitude, 100 seconds.

Relative Amplitude of Action Potential
MUSCLE IRRADIATION STUDY

T = 15°C

SOUND PULSE - 19 ATMOSPHERES PEAK, 60 SECONDS

Figure 18
Figure 19

Relative Amplitude of Action Potential

Stimulation Number

IRRADIATION STUDY - Time between stimulation 5.5 seconds - Temp. 15°C.
Sound Pulse - 19 Atmospheres peak amplitude, 60 seconds.
only about 75 percent suppression was obtained, with some fibers still active after the irradiation. It took a 50 second pulse of sound at 19 atmospheres to obtain this result. This run, in conjunction with some others made at lower temperatures, indicates that the effect of the sound on the muscle is definitely temperature dependent.

Any consideration of medical applications requires more complete information on dosage required to produce a change in the muscle contraction (dosage in this case is defined as the pressure amplitude and time of irradiation required to produce an observed effect). Such information is also necessary in evaluating any physical mechanism that is proposed to explain the experimental results. Data for a curve of minimum dosage required to produce a change in the electrical conduction of the biceps muscles was, therefore, obtained. It was not attempted to obtain dosage data for complete block of the propagated action potential, since it was found that such data was quantitatively somewhat unstable. There are a number of possible physical explanations for this, besides perhaps some biological ones. Reference to the beam patterns of the sound field indicates that there may be as much as a ten percent variation of the sound pressure amplitude over the muscle if it is centered in the field. This variation is even greater if the muscle is off center by the possible error of a millimeter. Since the voltage threshold of the effect is very sharp - for a 30 second irradiation pulse a change of 25 percent in the sound pressure amplitude carries the experiment from no effect to complete block - consistency in quantitative measurements is difficult to attain. It was therefore decided to obtain dosage data for minimum effects.
A minimum effect was quantitatively defined in two different fashions. For the preliminary study carried out on crystal system one, the minimum effect was defined in the following manner. For a fixed irradiation time, runs were carried out for various crystal voltages spaced about 250 volts apart. The point for minimum effect for that time was defined to lie between the sound pressure amplitude corresponding to the crystal voltage at which at least three muscles in a row were not permanently effected by the sound and the sound pressure amplitude at which at least one muscle suffered a permanent decrease in action potential of more than ten percent. When the reciprocal of the irradiation time was plotted against the sound pressure amplitude for minimum effect, a straight line resulted. This is in accord with the curve obtained by Fry from data taken on the paralysis of frogs irradiated in the lumbar enlargement region of the spinal cord.

A more extensive set of experiments was carried out on crystal system two to obtain a minimum dosage curve. For each fixed irradiation time, three muscles were irradiated at each of several crystal voltages spaced 250 volts apart. The point for minimum effect was defined to lie between the pressure amplitude at which at least two of the three muscles had their action potentials reduced more than ten percent and that pressure amplitude at which no more than one muscle was so affected. A further proviso was added that at higher pressure amplitudes than the first, a greater reduction in action potential must be observed, and at pressure amplitudes below the second the observed effects must be smaller. The minimum dosage curve of Figure 20 was obtained from this data. The temperature at which all this data was obtained varied between 18 and 20 degrees Centigrade. Over eighty frogs were used in obtaining the
Figure 20

MINIMUM DOSAGE STUDY

T = 18° - 20° C

Peak Pressure Amplitude - Atmospheres

1 x 100 - Time in Seconds

30° Orientation

30°
data for this curve while about forty frogs were used to obtain the preliminary curve. The data for Figure 20 took a month to obtain. The stability of the crystal system two over this period of time is illustrated in Figure 21 where the peak output of the barium titanate probe, in a sound field produced by applying 500 volts to the crystal, is plotted as a function of time.

It was observed in the course of the experimentation that no differences in effects occurred by switching the muscle end for end. The question of structural or geometric orientation of the observed effects naturally arose. As a result of this, two of the points obtained for the curve of Figure 20 were repeated with the muscle oriented at 30 degrees to the crystal rather than parallel to the crystal. These results, as shown in Figure 20, demonstrate that there is essentially no difference for the oriented muscles.

To complete the picture of the effects of sound on the excised biceps muscle, some histological studies were carried out of sections stained with hematoxylin-eosin. Figure 22 shows a typical control and a typical irradiated preparation under low magnification. Figure 23 shows the irradiated preparation under higher magnification. The control and irradiated muscles were handled identically except for the application of the sound. Electrical measurements on the irradiated muscle pictured showed complete block of the action potential due to the sound. No differences were observed microscopically between the irradiated and control tissue. Observations of the sections of irradiated tissue indicate no tissue tearing or vacuolization.

Some studies being carried out on the irradiation of the gastrocnemius muscle of tree frogs (see Fig. 5) with the vascular
SYSTEM STABILITY STUDY - CRYSTAL SYSTEM NO. 2

500 Volts on Crystal, Barium Titanate Probe
CONTROL MUSCLE - LOW MAGNIFICATION

IRRADIATED MUSCLE - LOW MAGNIFICATION

Figure 22
IRRADIATED MUSCLE - HIGH MAGNIFICATION

Figure 23
system intact, indicate that similar effects (large reduction of the action potential height) can be obtained on such preparations.
TEMPERATURE CHANGES DUE TO SOUND ABSORPTION IN THE MUSCLE

Once it was established that high frequency, high intensity sound blocked the propagation of the muscle action potential, it became necessary to investigate possible mechanisms involved in the phenomenon. Since there is a widespread tendency to consider tissue heating as the basic cause of ultrasonic biological effects, the measurement of muscle heating was the next problem undertaken.

Temperature changes in irradiated muscles were measured by means of imbedded thermocouples. These thermocouples are made of three mil copper and constantan wires joined together with a soldered lap joint. The thermocouple being used was threaded through the excised, mounted muscle and connected to a Rubicon type B potentiometer. Equilibrium measurements were used. The temperature in a muscle was found to come to equilibrium in less than ten seconds after the sound was turned on and voltage readings were taken for sound durations of 35 seconds and 60 seconds in alternate runs. There appeared to be no differences due to the different sound durations. It was always attempted, when placing the thermocouple in the muscle, to place it as close to the center line as possible. The peak of the sound field was located with the barium titanate probe. A pointer was then fixed opposite the probe, the probe was removed, and the muscle was located opposite the pointer with the thermocouple junction at the center of the field. This latter placement was possible because the muscle is translucent. Voltage measurements were taken of the thermocouple output for various
sound levels so that a curve could be obtained of the temperature change as a function of sound level. The thermocouple was calibrated for each muscle while still in the muscle. The holder with muscle and imbedded thermocouple was placed in baths of Ringer's solution at various temperatures and output voltage readings were taken. The temperatures of the baths were measured with a mercury thermometer and calibration curves of output voltage as a function of temperature were plotted from this.

Data of the type described were taken on ten muscles. A typical calibration curve and a temperature measurement curve are shown in Figure 24. In eight of the ten sets of data, readings were taken for sound intensities up to about 120 watts/cm². In all but one of these cases, a plot of temperature rise as a function of the square of the crystal voltage showed a linear relationship. The one set of non-linear data was, therefore, disregarded as representing an atypical situation, exhibiting possible cavitation at the thermocouple-muscle interface, and casting no light on the problem of temperature rise in the muscle in the absence of a thermocouple. Table 2 shows values of temperature rise obtained from the data curves for peak sound intensities of 123 and 94 watts/cm². The higher level is the one at which it is possible to completely block the muscle response in about 30 seconds. At the lower level it takes about 100 seconds to accomplish this. These values are for a temperature of about 22° Centigrade. It is apparent from Table 2 that there is quite a bit of scatter of the data. The reasons for this are more readily understood in terms of the theoretical aspects of the problem and, therefore, a discussion of this scatter will be presented after a theoretical analysis.
TEMPERATURE CHANGE IN MUSCLE UNDER SOUND IRRADIATION

Calibration
δ = -0.041 mv/°C
TABLE 2: MEASURED TEMPERATURE CHANGES ON EXCISED BICEPS MUSCLE UNDER SOUND IRRADIATION

<table>
<thead>
<tr>
<th>Muscle Number</th>
<th>Temperature Change at 94 watts/cm² °C</th>
<th>Temperature Change at 123 watts/cm² °C</th>
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</thead>
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<tr>
<td>1</td>
<td>5.5</td>
<td>7.1</td>
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<td>2</td>
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<tr>
<td>4</td>
<td>4.3</td>
<td>5.6</td>
</tr>
<tr>
<td>5</td>
<td>3.2</td>
<td>4.2</td>
</tr>
<tr>
<td>6</td>
<td>3.4</td>
<td>4.4</td>
</tr>
<tr>
<td>7</td>
<td>4.6</td>
<td>6.0</td>
</tr>
<tr>
<td>8</td>
<td>5.3</td>
<td>6.9</td>
</tr>
<tr>
<td>9</td>
<td>9.6</td>
<td>12.5</td>
</tr>
</tbody>
</table>

When equilibrium conditions of temperature exist, the general heat flow equation for a body in which heat is being generated internally can be expressed in the form:

\[ k \nabla T + A = 0 \] (4.1)

where \( k \) = thermal conductivity of the medium

\( T \) = temperature

\( A \) = heat per unit volume being generated per second at any point in the medium.

Equation (4.1) can be solved in a variety of ways depending upon the physical boundary conditions of the problem. In the problem being considered, the muscle is roughly the shape of a 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 ten percent.
over the muscle. In the axial direction, the beam is about seven millimeters 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 from the physical conditions. One is an infinite solid, circular cylinder with a constant amount of heat generated throughout and 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 generated throughout and the surface kept at a constant temperature. The first picture will probably lead to predicted temperature values in the muscle which are high, since heat is not actually being supplied all along the cylinder but only in a small region. The second picture will probably lead to low values of predicted temperature rise, since part of the boundary is then not Ringer's solution but is muscle and it will not remain at the fixed temperature of the Ringer's solution.

Considering the cylindrical situation, equation (4.1) becomes:

$$\frac{d^2T}{dr^2} + \frac{1}{r} \frac{dT}{dr} = - \frac{A}{k} \ . \quad (4.2)$$

Upon solving for $T$,

$$T = \left(- \frac{A}{k}\right) \frac{r^2}{4} + C_1 \ln r + C_2 \ . \quad (4.3)$$

The boundary conditions for the problem are:

$$r = 0, \quad T \neq \infty$$

$$r = a, \quad T = T_1 \ .$$

The solution, therefore, is:

$$T - T_1 = \frac{A}{4k} (a^2 - r^2) \ . \quad (4.4)$$
If the spherical situation is considered then equation (4.1) becomes:

\[
\frac{d^2T}{dr^2} + \frac{2}{r} \frac{dT}{dr} = -\frac{A}{k}. \tag{4.5}
\]

Upon solving for \(T\),

\[
T = \left( -\frac{A}{k} \right) \frac{r^2}{6} + \frac{C_1}{r} + C_2. \tag{4.6}
\]

The boundary conditions in this case are:

\[\begin{align*}
& r = 0, \quad T \neq \infty \\
& r = a, \quad T = T_1
\end{align*}\]

hence this solution is

\[
T - T_1 = \frac{A}{6k} (a^2 - r^2). \tag{4.7}
\]

Equations (4.4) and (4.7) demonstrate that the solutions for temperature distribution as a function of radial distance are identical in form and differ at any point by only 33 percent in magnitude. Since these were essentially extreme situations with the true physical picture lying somewhere between, it seems reasonable that the form of solution very closely approximates the actual situation in the muscle. A test of the accuracy of the magnitudes of temperature predicted by the equations can be made by inserting appropriate constants and comparing the results with the values measured with thermocouples.

The value of \(A\), the heat generated per second at any point, is given by

\[
A = I \mu \tag{4.8}
\]

where \(I = \text{sound intensity}\)

\(\mu = \text{absorption coefficient}\)
In order to obtain values corresponding to Table 2, intensities of 123 and 94 watts/cm² were used. The value of $\mu$ for striated frog muscle was found by Fry⁴ to be about 0.2. For lack of more information, the value of the thermal conductivity of water ($k = 0.00596$ watts/cm °C at 20°C) was used in the calculations. An examination of the tabulated values of the diameter of 50 muscles as indicated on Table 3 demonstrates that a value of $a = 0.1$ cm. is reasonable to use. Table 4 shows the computed values of peak temperature rise ($\gamma = 0$) for the two geometries considered at the two sound intensities considered.

**TABLE 3: MUSCLE DIAMETER MEASUREMENTS**

<table>
<thead>
<tr>
<th>Number of Muscles</th>
<th>Muscle Diameter (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.2</td>
</tr>
<tr>
<td>7</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>1.7</td>
</tr>
<tr>
<td>24</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**TABLE 4: COMPUTED TEMPERATURE CHANGES ALONG THE AXIS OF A MODEL OF A MUSCLE UNDER SOUND IRRADIATION**

<table>
<thead>
<tr>
<th>Intensity watts/cm²</th>
<th>Temperature Rise Cylinder Geometry °C</th>
<th>Temperature Rise Spherical Geometry °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>123</td>
<td>10.3</td>
<td>6.9</td>
</tr>
<tr>
<td>94</td>
<td>7.9</td>
<td>5.3</td>
</tr>
</tbody>
</table>

A comparison of Tables 2 and 4 demonstrates that the agreement between theory and measurement is good. An examination of
equations (4.4) and (4.7) helps explain why there are some differences and why there is scatter in the measured values. Some of the difference may be accounted for by inaccurate positioning of the thermocouple. If the thermocouple is half the radius away from the axis of the muscle, then the reading will be only three quarters of the peak value. Much of the difference can be accounted for by variation of the muscle size. Since the temperature rise is proportional to the square of the muscle radius, an increase in this value of only 25 percent leads to a change of temperature rise of more than 50 percent. These two factors would therefore tend to account for the differences between theory and measurement and the scatter in the measured values.

A pertinent point to note from the equations is that the temperature rise at any radial distance is proportional to the area outside that distance, therefore, half the muscle fibres undergo a temperature rise of less than half the peak value.
CHAPTER V

EFFECTS OF EQUILIBRIUM TEMPERATURE CHANGES ON MUSCLE ACTION POTENTIAL

With the temperature change in the muscle due to sound irradiation quantitatively established, it is important to discover just what effects this change can have on the muscle action potential and how it influences the interpretation of the effects of the sound irradiations. This problem has not often been considered previously. Early work by Bernstein, Gad and Heymans, and Doi involved temperature effects on the physiology of muscle but they dealt entirely with the effects of temperature change on the mechanical tensions developed by the muscle. Some of their evidence is contradictory, and furthermore, it is at present difficult to draw conclusions about the electrical properties of muscle from mechanical measurements. More recently, Walker made some measurements on both mechanical tensions and electrical action potentials in muscles. His work was mostly in vivo on rats and frogs. What work he did on isolated frog muscle was mainly with mechanical measurements. His measurements on the heights of action potentials in rat muscle as a function of temperature indicates that the height increases and then decreases as the temperature is reduced from 37°C to 22°C. His measurements on the duration of the action potential indicate that it increases with decreasing temperature.

Since the evidence of previous investigators is sparse and not very conclusive on various points, experiments were undertaken to measure the effects of equilibrium temperature change on the
frog muscle action potential under conditions similar to those established for the sound irradiation experiments. Tests were carried out on seventeen frog hind leg biceps muscles. These muscles were mounted in the same holder that was used for the sound irradiation experiments and the electrodes were threaded through the muscle in the same fashion. The tests were performed with the muscles suspended in frog Ringer's solution with the sodium bicarbonate omitted. The concentration of tubocurarine chloride solution used to eliminate possible nerve-muscle excitation was the same as that used in the sound irradiation experiments. Two types of runs were made. In one the muscle was kept in the same saline-curarine solution throughout the entire experiment and the temperature of the bath was varied by heating on a hot plate or cooling on ice. Electrical measurements were made periodically. Of ten muscles carried through this process, only two showed complete block of the action potential. One such block occurred when a muscle was taken to greater than 40°C and the other occurred when a muscle was taken below 10°C. In all other cases, varying the temperature between 15°C and 35°C produced changes in the height and width (duration) of the propagated action potential but not complete block.

Seven muscles were carried through a different type of run. A number of saline-curarine baths were kept at various fixed temperatures and the muscle was transferred to the different baths. The muscle was kept in each bath for two minutes to bring about equilibrium conditions before electrical measurements were made. This method of measurement was found to lead to the most consistent results. In six of the seven muscles, almost complete reversibility in height and width of the action potential was measured as the
muscle was carried through temperatures from about 20°C to about 35°C and then back to 20°C. In no case did complete blocking occur. Pictures of the action potentials recorded during one of these runs are shown in Figure 25. Figures 26 to 32 show quantitatively the relative changes in height and width of the action potential as a function of temperature for the seven muscles in this set. It is interesting to note from these curves that since both the height and width (duration) of the action potential decrease with increasing temperature, the total charge involved in the action potential decreases. This might indicate that the excitability of muscles under electrical stimulation (for example, nerve-muscle stimulation) is very much a function of temperature. An examination of Figure 25 indicates that the time delay between the artifact and the action potential response decreases quite a bit with increasing temperature. This would indicate that the velocity of conduction of the action potential probably increases with increasing temperature with perhaps some decrease in latency time occurring.

More pertinent to the problem at hand, an examination of Figures 26 to 32 indicates that while the height of the action potential decreases as the temperature is raised from 20°C to 35°C, complete block does not occur in this range of temperature and furthermore, the process is reversible as long as the temperature does not increase out of this range. Therefore, as long as the sound absorption in the muscle does not lead to a temperature greater than 35°C throughout the muscle, complete and irreversible block of the action potential cannot be attributed to the temperature change.
TEMPERATURE STUDY

Figure 25
Figure 26
TEMPERATURE EFFECT ON ACTION POTENTIAL - TM2
Figure 28

TEMPERATURE EFFECT ON ACTION POTENTIAL - TM3
TEMPERATURE EFFECT ON ACTION POTENTIAL - TM4
Figure 30
Figure 31

TEMPERATURE EFFECT ON ACTION POTENTIAL - TM6
CHAPTER VI

ANALYSIS OF THE EXPERIMENTAL RESULTS

Once it is definitely established that high intensity, high frequency sound can greatly reduce or completely block the propagated electrical action potential that is a comcomitant of muscle twitch contraction, it becomes necessary to analyze the conditions of the experiment to see what conclusions can be drawn as to the causes of the observed effects. There appear to be at least five classifications into which the possible mechanisms can fit. The first two are in the nature of artifacts since they depend upon the specific experimental conditions. It is possible that the hydrodynamical flow, which is at all times present in a sound field in fluids, can be causing mechanical difficulties at the connection of the electrodes to the muscle. In addition, it is possible that direct mechanical forces on the electrodes (radiation pressure, etc.) can be causing the observed effects. Besides these artifacts, the effect of the temperature rise in the tissue due to the absorption of sound may be responsible for the observations. Many effects of sound on biological systems are caused by heating, and it therefore becomes important to establish whether or not this one is too. A fourth classification is the cavitation effect sometimes occurring when negative pressures are present in a fluid due to the high amplitude of the sound. Such negative pressures were certainly present in the experiments on excised muscles. The final possibility, and the most interesting from the point of view of uniqueness in the method of production by sound, is that mechanical
forces which are inherent in the sound field travelling through a medium are causing destruction in the tissue. These forces can be either unidirectional such as radiation pressure or Oseen type forces, or sinusoidally time varying forces such as are due to the pressure amplitude of the sound wave or viscous effects on particles. It will be the aim of this analysis to show that it is unlikely that the observed effects are caused by any of the first four mechanisms and that they therefore probably fit in the last classification.

In investigating the flow artifact possibility, experiments were carried out with hydrodynamic flows produced by running fluid into a jet immersed in the coupling liquid and directed at the muscle. A diagram of the experimental system is shown in Figure 33.

![Diagram of Flow Artifact Testing Equipment](image)

**Figure 33**

Flow velocities much higher than those normally produced by the sound field were used. In order to establish the ratio of the
fluid velocity under jet conditions to that produced by the sound field, approximate velocities were obtained by injecting small resin particles into the flowing fluid and measuring the time it took them to move about six inches. The results of the flow artifact tests are indicated in Table 5. It is apparent from the table that even the very rapid flow being used in the tests had minor effects on the height of the observed action potential, and that the results of the ultrasonic irradiations (very large reduction or complete block of the action potential) were not caused by a flow artifact.

In eliminating the possibility of direct electrode artifacts, two types of experiments were carried out. In the first of these, a balsa wood fixture was mounted on the muscle holder for the purpose of blocking the electrodes from the direct sound field. As

<table>
<thead>
<tr>
<th>Muscle No.</th>
<th>Run No.</th>
<th>Approximate Flow Velocity</th>
<th>Flow Time (Sec.)</th>
<th>Temp. (°C)</th>
<th>Percent Permanent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>6 at start</td>
<td>30</td>
<td>19.5</td>
<td>-12</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>6 at start</td>
<td>30</td>
<td>19.5</td>
<td>+10</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>6 at start</td>
<td>30</td>
<td>19.5</td>
<td>-4</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>6 at start</td>
<td>30</td>
<td>19.5</td>
<td>-1</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>9</td>
<td>30</td>
<td>19</td>
<td>-6</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>9</td>
<td>30</td>
<td>19</td>
<td>-2</td>
</tr>
<tr>
<td>4</td>
<td>a</td>
<td>6</td>
<td>30</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>6</td>
<td>30</td>
<td>18</td>
<td>-15</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>7</td>
<td>30</td>
<td>19</td>
<td>+14</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>7</td>
<td>30</td>
<td>19</td>
<td>-19</td>
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<tr>
<td>6</td>
<td>a</td>
<td>6</td>
<td>30</td>
<td>19</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>6</td>
<td>30</td>
<td>19</td>
<td>-4</td>
</tr>
<tr>
<td>7</td>
<td>a</td>
<td>6</td>
<td>30</td>
<td>20</td>
<td>+1</td>
</tr>
</tbody>
</table>
is shown in Figure 34, it was still possible under these conditions to reduce the height of the action potential greatly with ultrasonic irradiation. In the second experiment, a pair of electrodes was placed at the peak of the sound field and irradiated with a sound level equivalent to that which is normally on the electrodes under the customary experimental conditions. This level was determined from the geometry of the system and the relative sound field graph and was found to be a peak pressure amplitude of about half the normal value. Table 6 shows the results of experiments on

<table>
<thead>
<tr>
<th>Muscle No.</th>
<th>Peak Pressure Amplitude Atmospheres</th>
<th>Irradiation Time Sec.</th>
<th>Temp. °C</th>
<th>Percent Permanent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.5</td>
<td>30</td>
<td>19.5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>9.4</td>
<td>30</td>
<td>19.5</td>
<td>-11</td>
</tr>
<tr>
<td>3</td>
<td>9.6</td>
<td>30</td>
<td>19.5</td>
<td>-5</td>
</tr>
</tbody>
</table>

three muscles carried out under the described conditions. It is apparent from both types of experiments that electrode artifacts do not explain the observed ultrasonic effects.

A number of pieces of experimental evidence can be tied together to eliminate the possibility that the observed effects were caused by excessive muscle heating. An examination of the irradiation data indicates that it was possible to block permanently or greatly reduce the action potential height operating from equilibrium temperatures of 20°C and 15°C with a peak sound pressure amplitude of 19 atmospheres (123 watts/cm²), and from an equilibrium temperature of 22.5°C with a peak sound pressure amplitude of 16.6 atmospheres (94 watts/cm²). If the measured values of
Figure 34.

Blocked Electrode Study - Time between stimulation 7 seconds, Temp. 18°C, Sound Pulse - 19 Atmospheres Peak Amplitude, 30 secs.
temperature change in the muscle under the same irradiation conditions are then taken into account, it can be seen that the temperature in the muscle does not exceed about 30°C, and in fact for the 15°C data this temperature does not exceed about 25°C. An examination of the effects on the muscle action potential of equilibrium temperatures up to about 35°C indicates that whatever suppressing effects these temperature changes have on the action potential height, they do not cause complete block and they are essentially reversible. The measured values of temperature change in the muscle are supported by calculated values, and the theoretical analysis further indicates that half the fibers in the muscle undergo temperature changes of less than half the maximum value. It is therefore even more unlikely that these fibers can be affected by heating.

The possibility has been raised that even if the average temperature rise in the tissue is not great, there still may be local hot spots at cellular interfaces. Fryl4 has disposed of this possibility by demonstrating that even if all the sound is absorbed at just a small percentage of the cellular interfaces, the temperature difference between the interfaces and the rest of the tissue would still be only a fraction of a degree.

In discussing the possible role of cavitation phenomena in the observed muscle effects, it should first be noted that as yet no direct attempt has been made to eliminate cavitation by carrying out the experiments under a hydrostatic pressure of greater than 20 atmospheres. There are a number of indications, though, that cavitation was not present to an appreciable extent under the experimental conditions which prevailed. The first such evidence
comes from an examination of the thermocouple measurements of temperature rise in the muscle under sonic irradiation. These measurements indicated that for peak sound pressure amplitudes up to about 20 atmospheres, in all but one experimental run, the temperature change measured was proportional to the square of the crystal voltage. Marked deviation from this linear relationship would be expected in the presence of cavitation. Furthermore, cavitation is more likely to occur in the muscle with the artificially introduced tissue-metal interface at the thermocouple than under normal irradiation conditions. Fry has demonstrated that sound level measurements in a medium undergoing cavitation become erratic. A second piece of evidence against excessive cavitation in the tissue under irradiation comes from an examination of the stained sections of irradiated muscle. Microscopic study shows no indication of vacuole formation or fiber tearing. A final argument against cavitation effects being important in these experiments is brought out by the work of Esche in which he found no evidence of cavitation present in beef muscle tissue up to sound intensities in the tissue of about 400 watts/cm².

It appears that flow artifact, electrode artifact, tissue heating, and cavitation are not likely mechanisms for explaining the observed phenomena. The effects of the high intensity sound irradiation of the muscle are therefore probably due to 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. Since the slope of the curve in this case appears to be about the same as that obtained from the
data on the paralysis of frogs irradiated in the lumbar enlargement region of the spinal cord, the mechanism may well be the same.

In attempting to establish a physical mechanism based on mechanical forces, in addition to an approximate empirical relation, it would be useful to know if the effect is geometrically random. The results for minimum dosage measurements were found to agree for the muscle oriented parallel to the crystal and for it oriented at $30^\circ$ to the crystal. The results were also the same for the muscle switched end for end. This would imply that either the phenomenon is geometrically random, or that it is of such a special geometry that it is in accord with the observations. It can be demonstrated that the latter possibility is unreasonable. Assume that there is a structure in the tissue oriented as shown in Figure 35. Assume further that there is damage to the structure under the

![Diagram](Figure 35)
effect of an axially directed minimum force $F_B$. When the direction of sound propagation is normal to the fiber, this force, $F_B$, is related to any force, $F_n$, caused by the sound field by the relation

$$F_B = F_n \sin \alpha .$$

(6.1)

For a sound field directed at the fiber at some angle $\theta$, this expression becomes

$$F_B = F_n \sin (\alpha + \theta) .$$

(6.2)

The experimental results indicate that for $\theta = 30^\circ$

$$F_n = F_n \quad \text{or} \quad \text{(6.3)}$$

$$\sin \alpha = \sin (\alpha + \theta) ,$$

(6.4)

For $\theta = 30^\circ$ this implies that $\alpha = 75^\circ$.

The results with the muscle switched end for end are equivalent to making $\theta = -30^\circ$ and imply that $\alpha = -75^\circ$. Since this is a contradiction, we can conclude that there is not the sensitive structural orientation originally assumed, or that the effects of the sound irradiation on the muscle are geometrically random.

The results of the minimum dosage study and of the orientation study should be useful in any attempt to validate a mechanical force mechanism.
CHAPTER VII

CONSIDERATION OF A MECHANICAL MECHANISM TO EXPLAIN SOME OF THE EFFECTS OF SOUND ON BIOLOGICAL SYSTEMS

In attempting to establish a physical mechanism to explain the non-thermal, non-cavitation effects of high intensity sound on muscle tissue or other biological systems, strong consideration must be given to mechanical force concepts. This is so since sound is a mechanical phenomenon and when it is propagated in a medium, various oscillatory and unidirectional forces result. If a sound wave in a fluid is oscillatory in nature, then any structure embedded in this fluid is subject to an oscillatory viscous force. In addition, because of the higher order effects due to the finite amplitude of the propagated wave, the structure will be acted upon by unidirectional forces such as radiation pressure, Oseen type forces, and mass streaming viscous forces.

Consider a particle suspended in a fluid in which a travelling wave of sound is propagated. The equation of motion of this particle under the influence of the linear viscous force due to the sound in the medium can be obtained from the differential equation

\[ M \ddot{R} + R \dot{r} = m \ddot{r} + R \dot{r} \]  \hspace{1cm} (7.1)

where \( \dot{r} \) = particle velocity of the medium
\( \ddot{r} \) = velocity of the particle
\( M \) = effective mass of the particle
\( m \) = effective mass of the displaced fluid
\( R \) = viscous force coefficient.
If an elastic restraint and a constant force due to the higher order effects are added then equation (7.1) becomes

\[ \ddot{r} + \frac{R}{M} \dot{r} + \frac{K}{M} r = \frac{m}{M} \ddot{\varphi} + \frac{R}{M} \dot{\varphi} + \frac{F}{M} \]  

(7.2)

where \( K \) = elastic constant

\( F \) = constant force.

If the particle velocity of the propagated wave is taken as

\[ \dot{\varphi} = U \sin(\omega t + \phi) \]  

(7.3)

in the region of the moving particle (this assumes that \( \gamma \) is small compared to the wavelength), then equation (7.2) can be solved to yield

\[ \varphi = C_1 e^{\alpha_1 t} + C_2 e^{\alpha_2 t} + A \cos \omega t + \frac{F}{K} \]  

(7.4)

where

\[ \alpha_1 = -\frac{R}{2M} + \sqrt{\frac{R^2}{4M^2} - \frac{K}{M}} \]

\[ \alpha_2 = -\frac{R}{2M} - \sqrt{\frac{R^2}{4M^2} - \frac{K}{M}} \]

\[ A = U \left[ \frac{m}{R} \sin \phi - \frac{1}{\omega} \cos \phi \right] \]

\[ \phi = \tan^{-1} \left[ \frac{\frac{m}{M} + (\frac{K}{M} - \omega^2) \frac{1}{\omega}}{(\frac{K}{M} - \omega^2) \frac{m}{R} - \frac{R}{M}} \right] \]

It shall now be assumed that the elastic restraining force is small, or quantitatively that

\[ \frac{K}{M} \ll \frac{R^2}{4M^2} \]  

(7.5)

Equation (7.4) can then be approximated by
\[ r = C_1 e^{-(K/R)t} + C_2 e^{-(R/R)t} + A \cos \omega t + \frac{F}{K}. \] (7.6)

The initial conditions can be assumed to be
\[ t = 0, \quad r = 0, \quad \dot{r} = 0. \]

Then
\[ C_1 + C_2 + A + \frac{F}{K} = 0 \quad \text{and} \quad K C_1 + R C_2 = 0. \] (7.7) (7.8)

The first decision that can be made from equation (7.8) is that
\[ |C_2| \ll |C_1| \quad \text{(see relation 7.5).} \] This leads to the conclusion that
\[ |C_2 e^{-(R/R)t}| \ll |C_1 e^{-(K/R)t}|. \] (7.9)

The mechanism that is being proposed is that the unidirectional force is great enough so that the resultant unidirectional motion of the particle carries it to a point where its elastic restraining member is stretched beyond normal limits and is permanently damaged. This deformation is assumed to be much greater than the amplitude of the oscillatory motion due to the linear viscous force. Under these conditions the approximate solution becomes
\[ r = A \cos \omega t + \frac{F}{K} \left(1 - e^{-(K/R)t}\right). \] (7.10)

Damage is assumed to occur when
\[ r_B = \frac{F}{K} \left(1 - e^{-(K/R)t_B}\right) \] (7.11)

where \[ r_B \gg A. \]
This equation can be solved for \( t_B \) (time after sound is turned on when damage occurs) so that

\[
t_B = -\frac{R}{K} \ln \left( 1 - \frac{r_B K}{f} \right).
\]  

(7.12)

It was previously indicated that '\( F' \), the unidirectional force, was due to higher order effects. For many of these it can be considered proportional to the square of the particle velocity amplitude.

\[
F = f U^2.
\]  

(7.13)

Hence

\[
t_B = -\frac{R}{K} \ln \left( 1 - \frac{r_B K}{fU^2} \right).
\]  

(7.14)

It is apparent from equations (7.11) and (7.12) that unless a certain threshold force is reached, damage never results. In terms of the particle velocity amplitude this threshold is

\[
U^2_0 = \frac{r_B K}{f}.
\]  

(7.15)

Equation (7.14) can be rewritten as

\[
\frac{R}{K} \ln \left( 1 - \frac{U^2_0}{U^2} \right)
\]  

(7.16)

or in terms of crystal voltage

\[
\frac{R}{K} \ln \left( 1 - \frac{V^2_0}{V^2} \right),
\]  

(7.17)

It is now possible to check the results of the theory with measurements that have been made on biological systems. From the muscle experiments, data is available of minimum dosage required for a ten percent reduction of the action potential (see Fig. 20). From some previous work by Fry\(^{14}\) there is data on the minimum
irradiation time for paralysis of frogs irradiated in the lumbar enlargement region of the spinal cord. According to equation (7.17) if the time for an effect is plotted against \(-\ln\left(1 - \frac{V_0^2}{V^2}\right)\), then the points should lie on a straight line through the origin. The slope of the resultant line is equal to \(\frac{R}{K}\). Figures 36 and 37 show the data plotted as indicated. In both cases the voltage corresponding to 100 seconds of irradiation time was used as the threshold value, \(V_0\). The agreement between theory and measurement appears to be quite reasonable.

The theoretical equations were derived with reference to a single particle. They are naturally meant to apply, in terms of effects, to a large number of similar particles. Some of the experimental work indicates that the effects are not geometrically oriented. There are a number of ways in which this observation can be in accord with the theory. If the constants \(r_B, K, \kappa, f\) are independent of geometry, then there would be no changes in results with changing directions of incident sound.

As another possibility, if each particle is constrained to move in only one direction but these directions are uniformly randomly distributed among the particles of interest, then a simple analysis demonstrates that a similar result is obtainable. Consider that an effect is observed when a fixed fraction, \(k\), of the structures under consideration is damaged. Corresponding to this fraction there is an angle, \(\Theta_o\), so that for orientation angles smaller than \(\Theta_o\) the structures are always damaged for a threshold voltage \(V_0\). For orientation angles greater than \(\Theta_o\), none of the structures is damaged at the threshold voltage. Then the only change in the analysis is that the threshold particle velocity
Figure 36

Muscle Data Plotted in Accordance with Theory $t_B = -\frac{R}{K} \ln\left(1 - \frac{V_0^2}{V^2}\right)$
Frog Paralysis Data Plotted in Accordance with Theory $t_B = -\left(\frac{R}{K}\right) \ln \left(1 - \frac{V_0^2}{V^2}\right)$

Hydrostatic Pressure - 1 Atm.  Temperature - 0-1°C.
amplitude becomes

\[ \nu_o^2 = \frac{v_B k}{f \cos \theta} . \]  

(7.18)

For angularly random directionality, \( \theta_o \) is related to \( k \) by the expression

\[ \theta_o = k \pi . \]  

(7.19)

If the general mechanism that has been advanced appears reasonable in the light of further experimental work, it can be elaborated to include factors like temporal random reorientation of particles and systems of a number of different types of particles.
CHAPTER VIII

CONCLUSIONS

The studies that have been discussed in this thesis demonstrate that under appropriate ultrasonic dosage conditions, the propagated action potential of the 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. Quantitative determination of the minimum dosage curve for a ten percent reduction of the action potential indicates that the shape of this curve is the same as the curve given by Fry\textsuperscript{14} for paralysis of the hind legs of frogs irradiated in the lumbar enlargement region of the spinal cord. Histological examination of stained tissue sections shows no gross tearing or vacuolization. Probe measurements made within the muscle also indicate that cavitation is absent. A theoretical investigation based on the action of unidirectional forces as the physical agent has been carried out, and the resultant model yields a relation which agrees in form with some quantitative measurements.

It is hoped that these studies will lead the way to future work on the effects of high intensity sound on muscle. It would appear that a reasonable next step would be to continue investigations of irradiation of muscle with an intact vascular system and irradiation of muscle in completely intact animals. In this way it can be seen whether the muscle will in time recover from the irradiation. Furthermore, histological time studies can be carried out to follow any tissue degeneration which might occur. If the
histological methods are not sensitive enough, chemical studies or physical chemistry studies of the irradiated tissue can be carried out.

The theoretical model that was advanced is interesting because of the agreement in form with some of the measurements. More experimental work must be carried out on multiple dose effects, standing wave effects, frequency effects, and effects on other tissues before its definite applicability can be determined. If future work indicates that the theory is reasonable, then it can be used to determine some of the mechanical constants of tissue structure.
BIBLIOGRAPHY


VITA

Walter Welkowitz was born on August 3, 1926 in Brooklyn, New York. He received the Bachelor of Science degree in Electrical Engineering from The Cooper Union in January, 1948. He began his graduate work at the University of Illinois at that time and received the Master of Science degree in Electrical Engineering from that institution in June, 1949. He held a Teaching Assistantship from February, 1948 to June, 1948; a Research Assistantship from June, 1948 to January, 1953; and a Research Associateship from January, 1953 to July, 1954. He served in the United States Navy from September, 1944 to June, 1946.