AN APPARATUS FOR STATIC STRENGTH MEASUREMENT OF SKELETAL MUSCLE IN VITRO

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F. TIMOTHY DRISCOLL

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ABSTRACT

AN APPARATUS FOR STATIC STRENGTH MEASUREMENT
OF SKELETAL MUSCLE IN VITRO

By

F. Timothy Driscoll

An apparatus was developed for measuring the static strength of the soleus and gastrocnemius muscles of the laboratory rat *in vitro*. The "Static Strength Analyzer" controls temperature, reference tension, stimulus input, and registers isometric tension in terms of grams on a digital readout. The overall accuracy of the system is ±0.3% of scale value.

A sample of twenty-one male albino rats (Sprague-Dawley) weighing 399 to 446 grams were tested and "optimal" values for muscle excitation were sought. Currents ranging from .1 ma to 15 ma and frequencies ranging from 40 cycles/second to 1,160 cycles/second were used. A 50% Duty Cycle was employed to insure a square wave impulse and the duration of stimulation time ranged from one to four seconds.

Due to the complexity of the problem and the sample size "optimal" values for muscle stimulation were not established.

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By

F. Timothy Driscoll

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DEDICATION

To Cindy whose sacrifice and understanding made it possible for me to advance my education.

ACKNOWLEDGMENTS

The author is indebted to Dr. William W. Heusner for his guidance throughout my course of study and to Robert L. Wells for his personal help and assistance on the design and the construction of the "Static Strength Analyzer."

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CHAPTER I

INTRODUCTION

At the present time, there is considerable interest in the differential effects of specific exercise programs on skeletal muscle. Research is being conducted on the laboratory rat and is being directed toward identifying the changes which occur in enzymes, capillaries, and muscle fibers (size and number). A factor which has not been adequately studied in relationship to these other factors, and is a specific effect of exercise, is strength. Strength, being an important physiologic variable, should be considered among the specific effects of different regimens of exercise.

Background

A review of the literature reveals several different types of strength testing apparatus for use with *in vivo* or *in vitro* muscle preparations (12,15,16,17). Investigators have controlled variables such as: temperature, pH, electrolye balance and oxygen supply. However, in most of the available studies the electrical parameters used for the stimulation of skeletal muscle are not clearly defined. When the parameters of stimulation are defined, the variability between studies is so great that few comparisons can be drawn.

Purpose of the Study

The purpose of this study was to build an apparatus for *in vitro* static strength measurement, which controls all significant variables, and to define the "optimal" electrical parameters for simulation of the rat's gastrocnemius and soleus muscle. Specifically, temperature, oxygen supply, stimulus input, pH, electrolye balance, and tension were controlled. Optimal values for current, frequency, and duration of stimulation were sought.

Scope of the Study

A "Static Strength Analyzer" was developed which controlls muscle tension, stimulus input, temperature, and oxygen supply and which registers isometric tension values in grams.

A sample of twenty-one male albino rats (Sprague-Dawley) weighing 399-446 grams were sacrificed and their soleus and gastrocnemius muscles excised. These muscles were then placed in a temperature-controlled bath of oxygenated Tyrodes solution for five minutes to allow for temperature stablization of the muscle. After the equilibration period, each muscle individually was stimulated and values of static tension were recorded.

Limitations of This Study

1. Size of the sample: due to the amount of time it took to develop and refine the instrumentation involved, the sample size was limited to twenty-one animals.

2. The electrical values derived from this study may not be applicable to studies incorporating nerve stimulation of the muscle or to studies of other skeletal muscles of the rat.

3. The amount of current that passes through the muscle is not controllable.

Definitions of Terms

Frequency.--Frequency is the number of electrical impulses received per second.

Current.--Current is the rate of flow of electrons through a circuit. In this case, it is used to artifically initate contraction of the muscle.

Duration of Stimulation.--Duration of Stimulation is the length of time the muscle receives stimulation.

Duty Cycle: Duty cycle is the percentage of time the current is flowing.

Static Strength.--Static strength is the force generated by a muscle during a single maximum contraction against an "immovable" resistance.

CHAPTER II

REVIEW OF LITERATURE

This review of literature is limited to experiments on electrical excitation of skeletal muscle for the purpose of determining isometric strength.

In the process of electrical stimulation of skeletal muscle, one must be concerned with five primary parameters: current, frequency, wave type, wave duration, and duration of stimulation. The reporting of the values of the parameters are of utmost importance if an interpretation of strength is to be made. Since the physiological effect occurring during electrical stimulation is determined by current, the reporting of voltage as a measure of stimulus strength is meaningless unless the resistance in the circuit also is defined (14,25).

A review of muscle-stimulation studies reveals an assorted number of values for voltage, ranging from three volts to thirty volts, with no mention of the resistance involved (2,3,4,5,7,8,11,20,21,22,23,24). The problem of interpretation is accented when one compares the results of Van Linge's study (24) with that of Binkhorst (3).

Van Linge found isometric tension values ranging from 680 grams to 1,150 grams for the plantaris muscle of 60- to 90-day-old trained rats. Binkhorst, using the plantaris muscle of 70-day-old trained rats, reported a mean value of 378 ± 96 grams for isometric tension. Van Linge reports no stimulation values, and Binkhorst reports only the wave type, wave duration, stimulation duration, and frequency. It is difficult to account for the great variability between their data.

Studies of the gastrocnemius muscle illustrate the same problem. Lund (12) tested static strength using 70-day-old trained rats and reported a mean value of 421 ± 22 grams. Thompson (21,22) also measured the isometric tension of the gastrocnemius and reported a mean value for untrained female rats, of approximately the same age, of 390 ± 70 grams. Schwartz (20) found a value of 1,356 ± 201 grams for the static strength of the gastrocnemius muscle of 24- to 31-day-old rats. Of these three, Lund is the only investigator who reported his stimulation in terms of amps and also defined his duration of stimulation and wave type. However, Lund did not give the frequency used. Thompson defined his stimulus as 30 volts at a frequency of 15 cycles/second with a square wave of .75 msec. Schwartz reported his stimulus as a "supramaximal" voltage at 120 cycles/second, for a five-second duration, using a square wave of .75 msec.

duration. Without all of the pertinent information, the data on gastrocnemius isometric tension are difficult to interpret.

Comparisons between studies of static strength data are not clear because of the variability in stimulation. Close (4,5), on stimulating the extensor digitorum longus of 4-week-old rats at a frequency of 300 Hz, reported a value of 50 $\pm$ 10 grams for isometric tension. He defined his stimulus strength as 15 volts/cm (refers to direct stimulation by a transverse electrical field in a bath fluid) for 0.2 or 0.3 msec. Gutmann (8) reported stimulating the extensor digitorum longus for 300-msec. duration, with a square wave pulse of 0.3 msec, at a frequency of 120 cycles/second. Using stimulation of "supramaximal" strength, Gutmann recorded the average peak tetanus tension to be 86.0 $\pm$ 4.3 grams for the extensor digitorum longus. Close (4,5) used the same stimulation values for the rat soleus as the rat extensor digitorum longus, except he found that a frequency of 250 Hz was needed to attain tetanic contraction of the soleus. Leach (11), also working with the rat's soleus, reported using a stimulation of five msec. duration with a frequency of 100 cycles/second and a voltage 20% above threshold for a one second duration to attain tetanic contraction of the soleus. Fex and Jirmanova (7), working with both the soleus and the flexor digitorum longus of the rat, defined their stimulus strength as 8-10 volts using a square wave pulse of 0.1 msec duration for a duration of 0.4 seconds.

Another example of the interpretation problem arising from the variability of muscle stimulation values can be seen when comparing the values Goldspink (9) and Walker (26) registered for the biceps brachii of the mouse. Goldspink reported maximum tension of 19 $\pm$ 2.0 grams and Walker reported 1,845 $\pm$ 121.7 grams. The reported stimulation accounts for some of the variation between these values. Goldspink stimulated with 50 volts of D.C. at a frequency of 50 cycles/second with a square wave of 100 msec duration. Walker stimulated with 50 volts A.C. and reported use of a frequency of 100 cycles/second to attain tetanic contraction. Rowe (18), although using the soleus of the mouse and stimulating via the nerve, defined his stimulus strength as 17.5 volts with a frequency of 200 cycles/second. He reported maximum tetanic tension values ranging from 13 grams for the control animal to 21 grams for the experimental animal.

Studies on the gastrocnemius muscle of guinea pigs are incomparable for the same reason. Schottilius (19), studying isometric contraction for use on force-velocity curves, defined his stimulus as "brief volleys of slightly supramaximal square wave pulses at a frequency of 105 cycles/second." Schottilius reported isometric tension values ranging from 621 $\pm$ 38 grams to 843 $\pm$ 52 grams. Barnard (1), studying the effect of exercise on the contractile properties of skeletal muscle, reported tetanic tension values of 2,504 ± 49 grams for control animals and 2,489 ± 82 grams for trained animals. Barnard defined his stimulation as a "supramaximal" voltage with frequencies of 45-50 cycles/second, which produced fused tetany. However, Barnard used frequencies of 100 and 150 cycles/second to determine maximum tetanic tension. A problem arises with the Wedensky phenomenon as reported in Truong's work (23). It seems that at frequencies which produce complete fusion, optimal tension is not developed. There is an optimal frequency at which incomplete fusion takes place where maximum tetanic tension is achieved.

A final example demonstrating the variability of reporting stimulation values is found in the work of Helander and Thulin (10) and Cullingham (6) on the cat. Helander and Thulin reported a stimulation strength of 3 to 6 volts at a frequency of 50 cycles/second would insure good tetanical fusion. Cullingham and collaborators defined their stimulus strength as 24 to 30 ma, a frequency of 120 to 160 cycles/second and rectangular pulses of 300 usec. These researchers did not report the duration of stimulation.

From these studies, it can be seen that a standard procedure should be implemented in reporting stimulus strength values in electrical excitation studies of skeletal muscle. This is of primary importance if progress is to be made in this field. With the present situation of great variability between studies, interpretation of the data is difficult.

CHAPTER III

METHODS

Testing Apparatus

The "Static Strength Analyzer" was developed to test the static strength of skeletal muscle in vitro (Figure 1). The structural components of the apparatus consist of (a) a plexigas console containing the electrical circuits of the apparatus (Figure 2), (b) a plexigas linear variable differential transformer mount, (c) an adjustable hemostat mount, (d) a plexigas bath divided into a Preparation Chamber and a Testing Chamber, and (e) two modified fish tank heaters.

The apparatus was built with the LVDT* mounted between two spring-steel blades to which a bolt-mounted hemostat was attached. The positioning of this system suspends the hemostat in the testing chamber. The mounting of the LVDT between two spring-steel blades with an aluminum extension carrying the bolt-mounted hemostat

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*A Schaevitz Model 100 DC-B linear variable differential transformer (LVDT) converts linear motion (full scale ± 0.1 inch) into a proportional DC signal with a linearity of 0.2% of scale value.

Figure 1. Static strength analyzer.

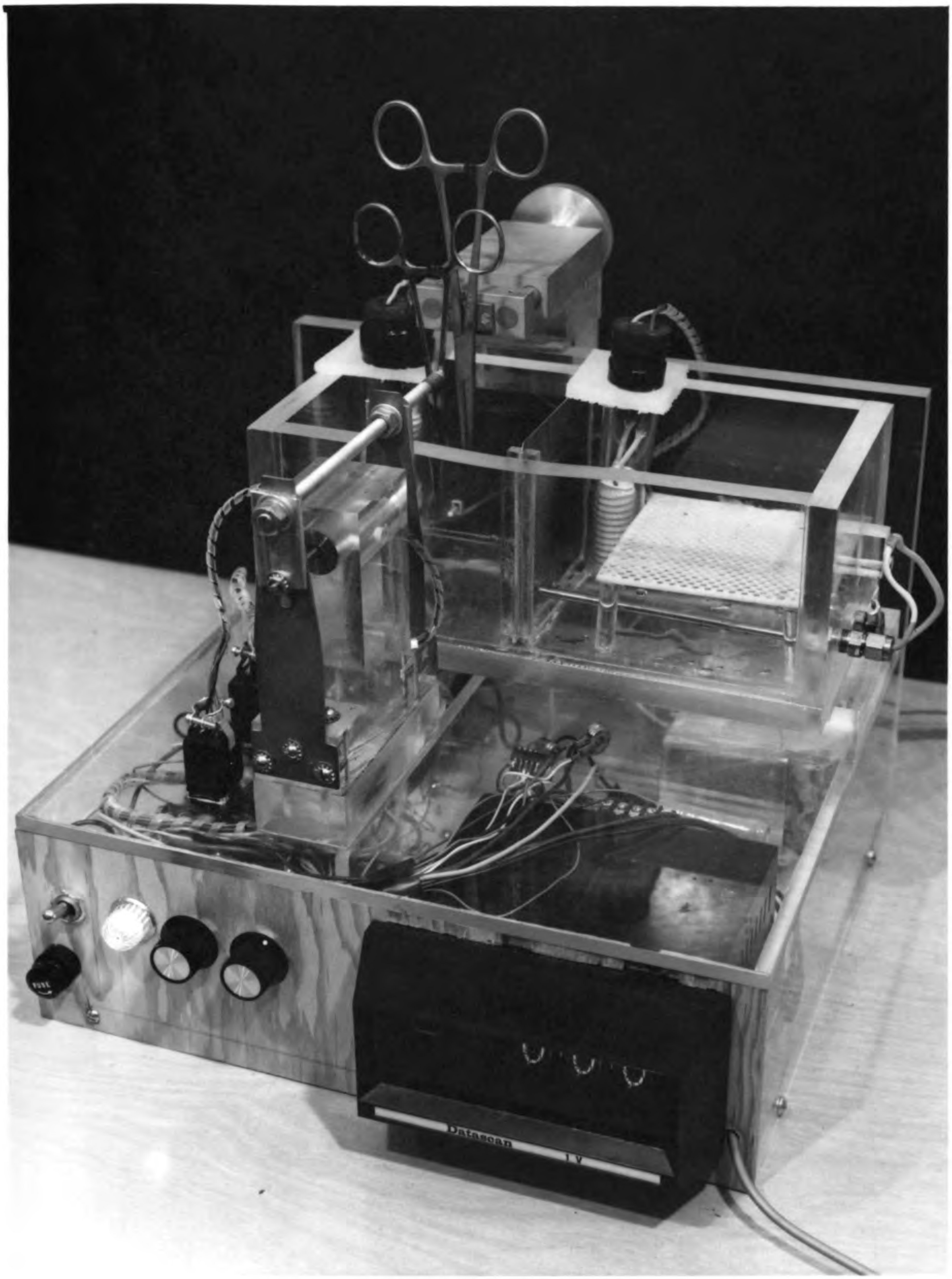
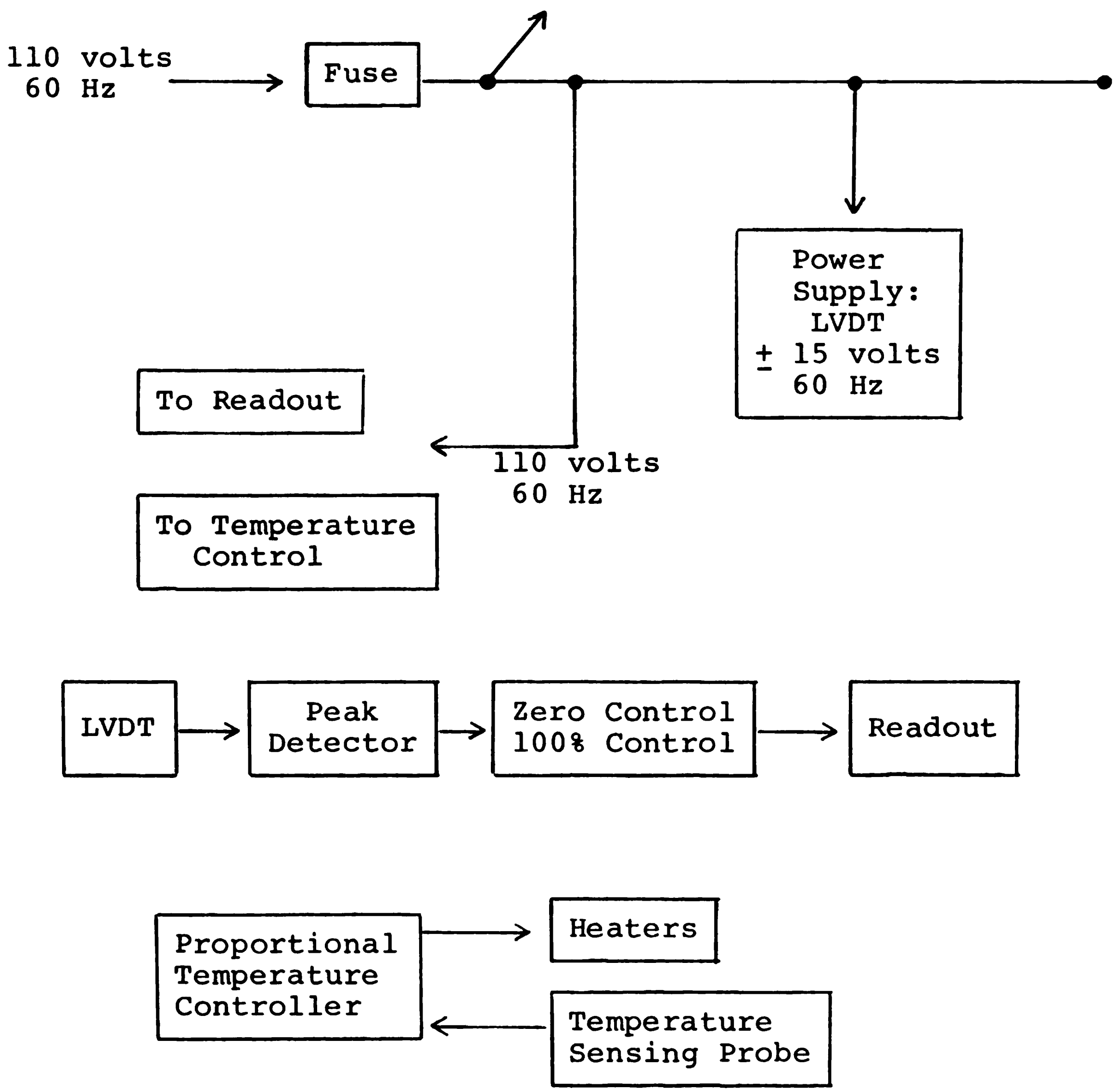

Datascan
LV

Figure 2. Block scheme of electrical circuits of "static strength analyzer."


110 volts
60 Hz
Fuse
Power
Supply:
LVDT
± 15 volts
60 Hz
To Readout
110 volts
60 Hz
To Temperature
Control
LVDT
Peak
Detector
Zero Control
100% Control
Readout
Proportional
Temperature
Controller
Heaters
Temperature
Sensing Probe

creates a rigid recoil system which transforms muscle contraction into linear displacement and resets the displaced core upon muscle relaxation. The LVDT output is displayed on a four-place digital readout in terms of grams of tension. The overall system has an accuracy of $\pm$ 0.3% of scale value.

The adjustable hemostat mount is a two-inch by four-inch aluminum block with a 40 tread shaft controlling the travel of a hemostat mounted on two spring loaded pistons. This system is mounted across from the LVDT with the hemostat suspended in the testing chamber. The adjustable hemostat controls the reference tension placed on the muscle and is capable of one gram steps of tension.

The bath is divided into two chambers, a Preparation Chamber and a Testing Chamber, which are temperature controlled at 37°C $\pm$ 0.1°C using a RFL Model 70 Proportional Controller (accurate $\pm$ 0.05°C of set point). The Preparation Chamber consists of a muscle platform and a gas system for bubbling a glucose solution (Tyrodes solution) with 95% oxygen and 5% carbon dioxide. The role of the preparation chamber is to allow muscle temperature equilibration and at the same time to keep the integrity of the muscle membrance, vessels, and nerves intact. The testing chamber contains mineral oil which acts as an electrical insulator around the muscle, thereby minimizing current leakage during direct muscle stimulation and supplying a temperature controlled medium.

The stimulus is supplied and controlled by the Grass Model S-4 Stimulator and applied directly to the muscle through the hemostats.

Measurement Procedure

Each animal was anesthetized with sodium pentobarbital and the soleus muscle was surgically removed by clipping the Achilles tendon and the origin of the muscle on the tibia. The gastrocnemius was surgically removed by clipping the Achilles tendon and severing the knee joint. The tibia was then cut below the origin of the two gastrocnemius heads on the tibial condolyes. This procedure allowed for the attachment of the two heads of the gastrocnemius to the adjustable hemostat without concern for adjusting the tension equally on both heads which would have been the case if the muscle had been removed from the bone.

Upon removal of the soleus and the gastrocnemius muscle, the muscles were placed in an environmental temperature controlled (37°C ± 0.1°) preparation chamber which contained Tyrodes solution (13) bubbled with 95% oxygen and 5% carbon dioxide. The muscles remained in this chamber for a period of five minutes to allow for temperature stabilization of the muscle.

After the temperature equilibration period, each individual muscle was moved to a temperature-controlled testing chamber of mineral oil. In the testing chamber,

the muscles were individually attached to the LVDT mounted hemostat and to the adjusted hemostat. Reference tensions of 2 grams and 5 grams then were placed on the soleus and gastrocnemius respectively. Each muscle was stimulated with different currents at different frequencies using a square wave. The range of currents used was from .1 ma to 15 ma and the range of frequencies used was 40 cycles/second to 1,160 cycles/second with approximately 20% step intervals (see appendix). The duty cycle was calculated for each frequency to insure a square wave stimulus. The duration of stimulation ranged from one second to four seconds.

During stimulation, responses were recorded on a Gilson recorder for analysis of duration of stimulation, and isometric tension values were recorded from the digital readout.

CHAPTER IV

RESULTS AND DISCUSSION

This study was undertaken to develop an apparatus for measurement of static strength of the laboratory rat's soleus and gastrocnemius muscles *in vitro*. An apparatus was built which controls temperature, reference tensions, stimulus input to the muscle, and records static strength in grams of tension.

The results of the data collected from twenty-one male albino rats (Sprague-Dawley, 399-446 grams) were minimal. The stimulus values producing the greatest static strength values are recorded in Table 1. However, due to the sample size and the complexity of the problem, the data were not statistically analyzed. Therefore, these values are subjective choices based on the production of the highest static strength values recorded for each combination of parameter values tested.

Although the data collected did not clearly define the "optimal" values of current, frequency, and duration of stimulation for muscle excitation, the data explains the variability of the results reported in Chapter II and

TABLE 1.--Parameter values producing the greatest static strength.

Current	Frequency	Duty Cycle	Duration of Stimulation	Static Strength
		Soleus		
.7-2ma	80 c/s	6.2 msec	2 sec.	74 grams
		Gastrocnemius		
2-3ma	300 c/s	1.6 msec	2 sec.	815 grams

points out the need for much more work in this area. This being the case, the specific effects of different exercise regimens will remain unclear until "optimal" values are established and a standard procedure for reporting stimulus strength is implemented.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

An apparatus for measuring static strength of the hind leg of the laboratory rat *in vitro* was developed. The "Static Strength Analyzer" controls temperature, reference tension, stimulus input, and registers isometric tension in terms of grams on a four-place digital readout. The overall accuracy of the system is 0.3% of scale value.

A sample of twenty-one male albino rats weighing 399 to 446 grams were tested and "optimal" values for muscle stimulation were sought.

Due to the complexity of the problem and the sample size involved "optimal" values for muscle stimulation were not established.

Recommendations

1. The use of higher currents than were used in this study should be investigated when trying to establish "optimal" values of skeletal muscle stimulation.

2. A study of the static strength response of skeletal muscle stimulated via the nerve as compared to values derived from direct massive stimulation of skeletal muscle should be undertaken.

3. In future studies, the muscle weight and fiber size and number should be established relative to static strength values.

4. The relationship of the concentration of myosin fibrils and the exercise regimens benefical to the development and/or structural changes of myosin fibrils should be investigated when studying static strength.

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APPENDICES

APPENDIX A

STIMULATION PARAMETERS

APPENDIX A

STIMULATION PARAMETERS

Current in mA: .1, .2, .3, .4, .5, .6, .7, .8, .9, 1, 2, 3, 4, 5, 6, 7, 8, 8, 9, 10, 11, 12, 13, 14, 15.

Frequencies	Duty Cycle	Frequencies	Duty Cycle
40 c/s	12.5 msec	240 c/s	2.1 msec
50 c/s	10.0 msec	290 c/s	1.7 msec
60 c/s	8.3 msec	350 c/s	1.4 msec
70 c/s	7.1 msec	420 c/s	1.2 msec
80 c/s	6.2 msec	470 c/s	1.1 msec
90 c/s	5.5 msec	570 c/s	.9 msec
100 c/s	5.0 msec	670 c/s	.75 msec
120 c/s	4.2 msec	800 c/s	.62 msec
140 c/s	3.6 msec	960 c/s	.52 msec
170 c/s	3.0 msec	1,160 c/s	.43 msec
200 c/s	2.5 msec		

Duration of Stimulation: one, two, three, and four seconds

APPENDIX B

RAW DATA SUMMARY CHART

APPENDIX B

RAW DATA SUMMARY CHART

c	.4	.5	.6	.7	.8	.9	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Isometric Tension Values for the Soleus																					
40 cycles/second																					
BWT																					
449	10	20	33	45	48	50	50	52	54	54	48[a] (R) (L)										
440				08	14	18	20[a] (R)														
60 cycles/second																					
BWT																					
449				36	40	38	35[a] (L)														
402	08	18			38	40	41	41[a] (L)													
440				00	13	10	07[a] (L)														
415				35	42	41	48	51[a] (L)													
412									39[b] (R)		50[b] (L)										
80 cycles/second																					
BWT																					
411				20	22	27	26	39			45[a] (L)					32	34	33	31	30[a] (R)	
AAA				71	71	69	65	70[a] (L)													
413				74	65	65	73[a] (R)														
399							51[b] (R)				36[b] (L)										
421											61[b] (R)				56[b] (L)						
120 cycles/second																					
BWT																					
435				26	31	28	31	43	47	46[a] (L)											
413											68[b] (L)										
445														61[b] (R)		64[b] (L)					

BWT									
200 cycles/second									
446			60[b] (R)						48[b] (L)
300 cycles/second									
443	15	30	41		44	38[a] (R)			
443	32	44	43		49	54[c] (L)			
350 cycles/second									
423	15	16	32		27		23[a] (R)		
423	00	32	18		24		28[d] (L)		
420 cycles/second									
439		02	10	11	13	14[a] (R)			
439		00	00	23	42	56[d] (L)			
570 cycles/second									
420	00		09		24		20	22[a] (R)	
420								11[b] (L)	

Note: Animal number four (BWT 404) was tested with the current held constant and the frequency varied.

Frequencies	420	600	1,160	300	200[e]
Current 2ma	39	15	15	30	28 (R)
Frequencies	1,160	600	420	200[e]	
Current 5ma	31	28	48	50 (L)	

APPENDIX B.--Continued

c	.4	.5	.6	.7	.8	.9	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Isometric Tension Values for the Gastrocnemius																					
									120 cycles/second												
BWT																					
402							002[f]	516	297	226[a] (L)											
413						215	110	489	468[a] (R)												
399										606[ab] (R)				544[bd] (L)							
421																			626[ab] (R)		683[bd] L
413																		661[bd] (L)			
									140 cycles/second												
BWT																					
415						041	055	392	492[a] (R)												
									200 cycles/second												
BWT																					
AAA						014	015	192	285	355[a] (R)											
404											259	AAA	469	576[a] (L)							
									300 cycles/second												
BWT																					
AAA						074	294	815	730[a] (L)												
412										633	376	260	525	454[a] (R)							
412										000	263	125	656	716[d] (L)							
445														701		643		555		486[a] (R)	

420 cycles/second												
BWT												
411						604	582	503	473	250[a] (R)		
411						275	275	354	412	442[d] (L)		
404	634	457	306	314	140[a] (R)							
470 cycles/second												
BWT												
443					478[a] (R)							
443					553[bd] (L)							
570 cycles/second												
BWT												
423						314		514		362	201[a] (R)	
423						302		388		447	600[d] (L)	
800 cycles/second												
BWT												
446				019		020		375		311		690[a] R
446				188		386		430		501		378[a] L
1,160 cycles/second												
BWT												
420	089		170		089		272		343[a] (R)			
420			029		047		214		360		450[d] (L)	

[a]Progressively tested in an ascending order left to right.
[b]The highest value obtained for the first stimulation with progressively lower values following.
[c]Current in milliamps.
[d]Progressively tested in a descending order right to left.
[e]Order of frequencies tested.
[f]Poor tissue connect.