A COMPARATIVE STUDY ON THE EFFECTS OF OUABAIN, RATTLESNAKE (*CROTALUS VIRIDIS VIRIDIS*) VENOM, AND MYOTOXIN *a* ON THE CONTRACTILE RESPONSE AND ULTRASTRUCTURE OF RAT SKELETAL MUSCLE

By

JAMES P. KNIGHT

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Thesis Approved:

Charlotte L. Dowby
Thesis Adviser

Thurlow E. Story

Calvin G. Barnes, Jr.

Norman N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

The venom of North America rattlesnakes contains both enzymatic and non-enzymatic proteins. The two most prominent symptoms of rattlesnake envenomation are hemorrhage and myonecrosis. Present antivenom treatment (antiserum) prevents death, but fails to inhibit local muscle degeneration (Homma & Tu, 1970; Minton, 1954; Stahnke, 1966). Recently, Cameron and Tu (1977) isolated a pure component (myotoxin a) from the venom of Crotalus viridis viridis (prairie rattlesnake). Myotoxin a is a basic (isoelectric point of 9.6) protein having a minimum molecular weight of 4,427 daltons (by amino acid composition) which composes about 18 percent of the crude venom. In vivo studies of the pathogenesis of myonecrosis produced by experimental injection of the crude venom (Stringer et al., 1971) and myotoxin a (Ownby et al., 1976) have been reported. Injection of the crude venom induces hemorrhage and muscle fiber degeneration within 3 hours, while myotoxin a produces varying degrees of dilatation of the sarcoplasmic reticulum and perinuclear space as well as myofilament breakdown from 3 to 48 hours. Ownby et al. (1976) proposed that the primary site of action of myotoxin a was either the sarcolemma or sarcoplasmic reticulum. Their hypothesis was that myotoxin a inhibited the Na-K ATPase located in the sarcolemma resulting in a net influx of sodium ions and subsequent influx of water. Swelling of the sarcoplasmic reticulum would be an early morphologic response to the toxin since the sarcoplasmic

reticulum serves as a reservoir for water. The pathogenesis of myonecrosis induced by myotoxin a is essentially the same as that caused by the crude venom, which indicates that myotoxin a is probably the main component of the crude venom causing myonecrosis. No physiologic data have been reported for myotoxin a; however, Vick (1966) has reported some physiologic effects from intravenous injection of lethal doses of crude rattlesnake venoms. Vick observed an immediate fall in arterial blood pressure and a decrease in heart rate upon injection. The mode of death appeared to be due to respiratory failure.

Morphologic effects, similar to those induced by crude venom and myotoxin a, have been observed after ouabain treatment. Swelling of the intracellular membrane compartments, especially the endoplasmic reticulum, was reported in isolated kidney tubules treated with ouabain (Ginn et al., 1968). Cardiac muscle treated with ouabain displayed projection of the nuclear membrane into the cytoplasm and an increase in amount of Golgi vesicles (Lindower & Marks, 1977). A contractile force study completed by Lüllman and Holland (1962) showed that an increase in contractile force was apparent with ouabain treatment. Several other reports have confirmed that ouabain acts on the Na-K ATPase system to give an increase in contractile response (Akera et al., 1970; Besch et al., 1969; Caldwell & Nash, 1977). However, the report by Rhee et al. (1976) indicated that ouabain acts on the Na-K ATPase to induce both increased contractile response at low concentrations and decreased contractile response at higher concentrations.

These studies prompted our research concerning the comparative effects of these agents on skeletal muscle. The specific objective of

this study was to determine and compare the effects of ouabain, crude venom, and myotoxin *a* on the contractile response and morphology of skeletal muscle *in vitro*.

CHAPTER II

MATERIALS AND METHODS

Forty-eight female rats (180-230 g) of the Wistar strain were obtained from Charles Rivers in two separate shipments for use in this study. Each shipment was divided into two replicates: one consisted of the lighter 12 rats and the other consisted of the heavier 12 rats. Each of the four replicates contained 11 treatments and 1 untreated physiologic control to give a total of 48 muscle preparations. A split plot design (Steel & Torrie, 1960) was used in which the main plots were the 11 treatments and the subplots were repeated measurements over time.

Muscle Preparation

The superficial digital flexor and gastrocnemius muscles were used in all experiments and were isolated in the following manner. The rats were anesthetized with ether, the skin was stripped away from the distal portion of the right leg, and a puncture was made between the calcanean tendon and deep digital flexor. The muscles were blunt-dissected proximally to the popliteal space where the posterior muscle bundle was cut free. After cutting the calcanean tendon the muscle bundle was quickly placed in a petri dish containing a standard solution of Minimum Essential Medium (MEM) which contained 1mM sodium pyruvate (Flow Chemical Company, 10-121) having a pH of 7.4 and osmolarity of 310 milliosmoles. The gastrocnemius and superficial digital flexor muscles were exposed by

removal of the deep pleural fascia, soleus and biceps femoris muscles. The exposed muscle preparation was weighed and only those preparations which weighed 0.9 to 1.2 g were used. Physiologic and morphologic observations were made on each preparation.

Agents Tested

The three agents were: ouabain, molecular weight 728.6, purchased from Sigma Chemical Company; crude Prairie rattlesnake (Crotalus viridis viridis) venom purchased from Miami Serpentarium in lyophilized form; and myotoxin a supplied by Dr. A. T. Tu, isolated by the procedure of Cameron and Tu (1977).

Standard concentrations of the above agents (ouabain, crude venom, and myotoxin a) were made by dissolving ten times the desired amount in MEM containing pyruvate. The final desired concentration was obtained by the addition of 1 ml of the above standards to the 9 ml bathing solution. The final doses (μg/gram muscle) used in this study were: ouabain: 0.015, 1.50, 15.0, 150.0; crude venom: 0.03, 3.0, 300.0; and myotoxin a: 0.015, 1.5, 15.0, 30.0. These doses of the above agents were selected to encompass both sublethal and lethal concentrations based on previous experiments (Ginn et al., 1968; Ownby et al., 1976; Vick et al., 1967).

Physiologic Studies

The distal end (calcanean tendon) of the muscle preparation, as described above, was tied to the base of a vessel containing 9 ml of MEM and pyruvate, bubbled continuously with 100 percent O_2, and maintained at 37°C. The proximal end of the muscle preparation was attached to a calibrated Narco Bio-system myograph type A which was connected to an

MK VI physiograph amplifier (E & M Instrument Company) for display. A constant 2 gram resting tension was applied while the muscle was directly stimulated with 130 V pulses of 0.5 second duration at 12 pulses per minute delivered from a MK V stimulator (E & M Instrument Company) via silver electrodes. A five-minute period of stimulation preceded addition of the agents tested, and this time was used as a control contractile period for each muscle preparation. The control contractile response ranged from 18 to 24 grams. Stimulation continued until the contractile response fell below 30 percent of the control contractile period. Aliquots of each bathing solution were retained for pH and osmolarity measurements. The response data for each experiment was standardized by averaging the responses in the control period and dividing that value into the average response for each minute following addition of the test agent. This ratio multiplied by 100 was considered as the percent contractile response in this study. Surface response diagrams were developed to exhibit any interaction between concentration and time with respect to percent contractile response. The data were analyzed by least squares as a split plot over time. The computer programming system used was Statistical Analysis System (Barr & Goodnight, 1972).

Morphologic Studies

To observe any variations in or artifacts induced by processing, samples (1-2 mm) from the soleus muscle were fixed immediately after removal from the animal. Additional samples were taken from the exterior 2 mm and central region of the remaining muscle preparation (gastrocnemius and superficial digital flexor) immediately after recording the physiologic data from each experiment. All the above samples were

immediately placed in 2 percent glutaraldehyde buffered with sodium cacodylate, pH 7.4 for 2 hours at 4°C. Overnight washing in cacodylate buffer containing sucrose was followed by secondary fixation in 1 percent osmium tetroxide for 2 hours, then dehydration in a series of graded ethyl alcohols. Propylene oxide was used as the transition solvent before embedding in Epon 812 (Luft, 1961). Thick sections (0.5-1.5 μm) were taken for light microscopic examination using a Sorvall MT-2 Ultramicrotome with glass knives and stained with Mallory's Azure II Methylene Blue (Richardson et al., 1960). Thick sections were viewed and only those cells in the early stages of degeneration were selected for electron microscopic examination. Thin sections (silver) were obtained using glass or diamond knives with an LKB Ultratome I and stained with aqueous uranyl acetate and lead citrate (Venable and Coggeshall, 1965) for observation in a Philips EM 200 electron microscope.

Light Microscopy

After all electron microscopic samples were removed, the remaining muscle was quickly placed into 10 percent phosphate buffered formalin for several days. After fixation these samples were washed in tap water, dehydrated in a graded ethanol series, cleared using chloroform and embedded in paraplast. Cross sections of the muscle (approximately 9 μm) were prepared by using an AO "820" microtome and stained with hemotoxylin and eosin for examination.

CHAPTER III

RESULTS

Physiologic Studies

Preliminary statistical analysis of the physiologic data indicated a difference between the responses of the untreated physiologic controls and the treated muscles. These controls were not used in the statistical comparison of the effects of the three agents. However, they were plotted on the response surface diagrams to indicate the normal decline in contractile response due to muscle fatigue. The evaluation of the response induced by the three agents made by least squares analysis of variance indicated a highly significant interaction due to time and dose within each agent tested (see Table I). Due to this interaction, separate statistical analysis was carried out on each agent and a surface for each agent was generated using the relationship between percent contractile response and two factors: dose and time (Figures 1, 2, and 3). A least squares fit was made in such a manner that the highest order polynomial in any term of the model was ≤ 2. The fitted surface, in general, would appear as follows:

$$Y_{ij} = B_0 + B_1X_1 + B_2X_2 + B_2X_1^2 + B_4X_2^2 + B_5X_1X_2$$

in which:

Y_{ij} = percent contractile response;

TABLE I

ANALYSIS OF VARIANCE ON CONTRACTILE RESPONSE

Source	Degrees of Freedom	Mean Square	Observed Significance Level (p)
Replicates	3	0.089680	0.1000
Agent	2	0.046270	0.3486
Dose (Agent)	8	0.092900	0.0563
Main Plot Error	30	0.042280	---
Time	9	1.006000	0.0001
Agent Time	18	0.006519	0.0001
Dose Time (Agent)	72	0.013290	0.0001
Subplot Error*	297	0.001340	---

*Coefficient of variance for main plot was 25%; for subplot, 4%.

Figure 1. Surface Response of Ouabain-Treated Muscles and Untreated Physiologic Control. All values plotted are means of the four replicates. Note all doses of ouabain produce a greater rate of decline of contractile response than the untreated muscles.

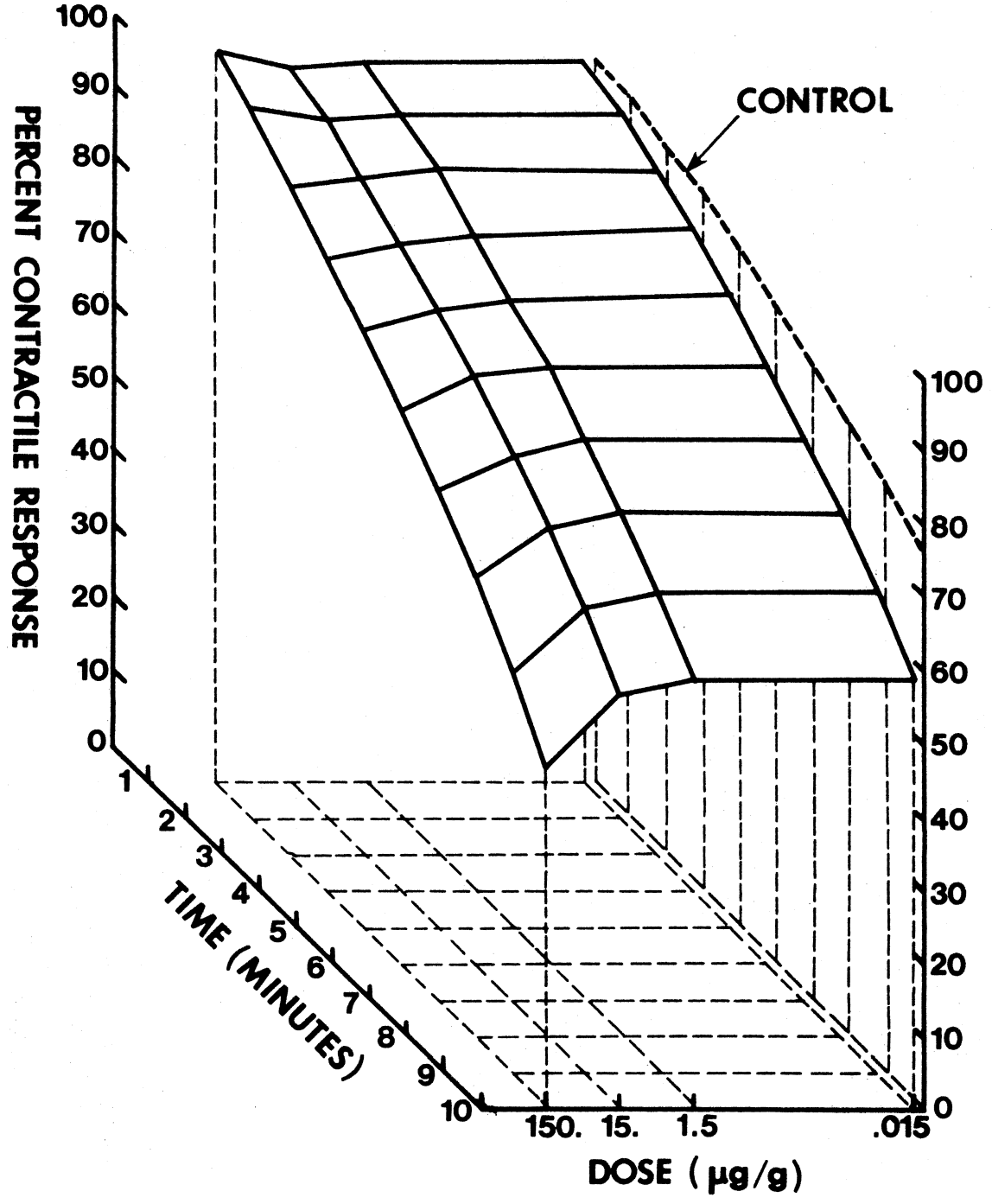

PERCENT CONTRACTILE RESPONSE
100
90
80
70
60
50
40
30
20
10
0
CONTROL
TIME (MINUTES)
1
2
3
4
5
6
7
8
9
10
150.
15.
1.5
.015
DOSE (µg/g)

Figure 2. Surface Response of Crude Venom (*Crotalus viridis viridis*)-Treated Muscle and Untreated Physiologic Control. All values plotted are means of the four replicates. Note all doses of crude venom produce a greater rate of decline of contractile response than the untreated muscles.

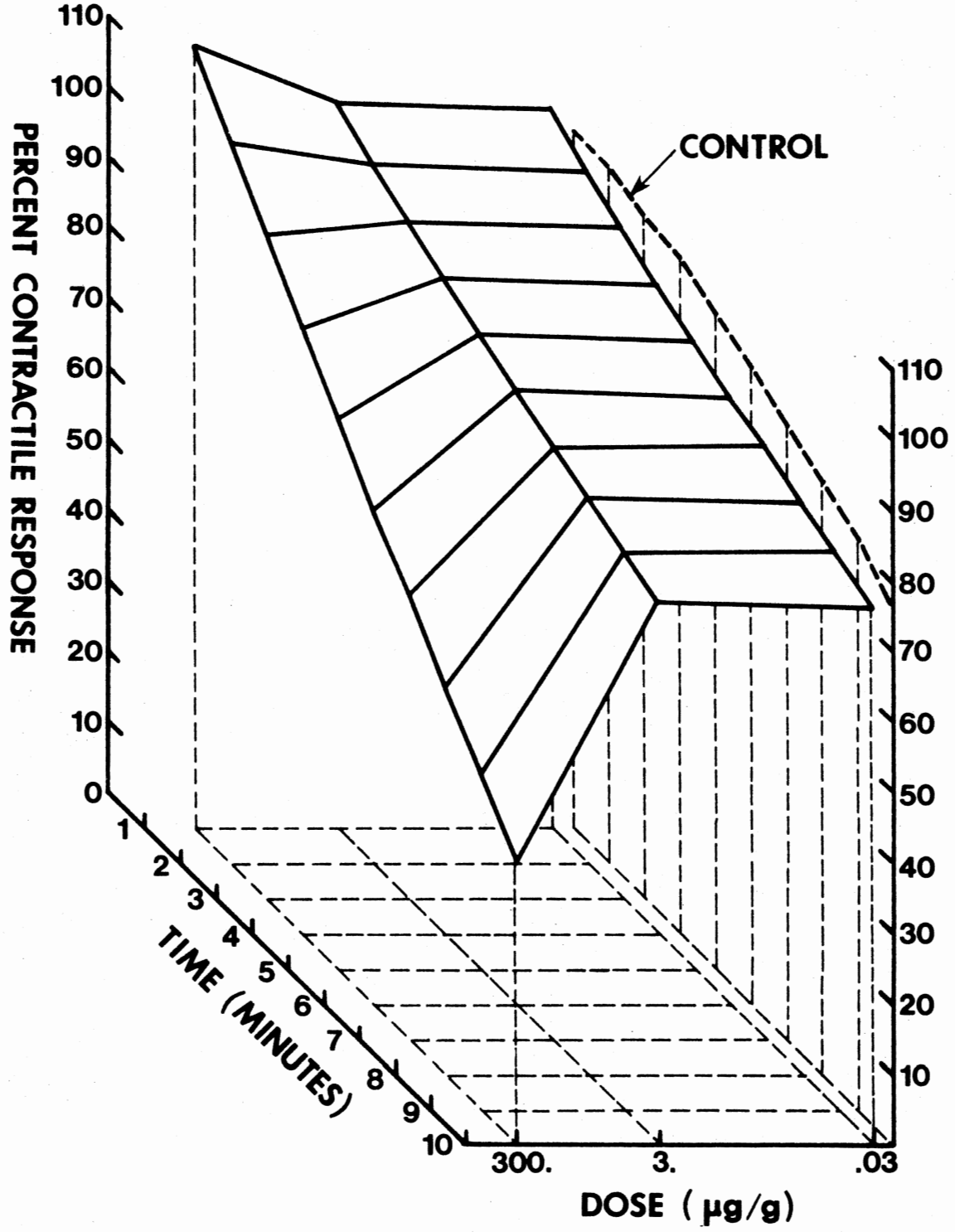
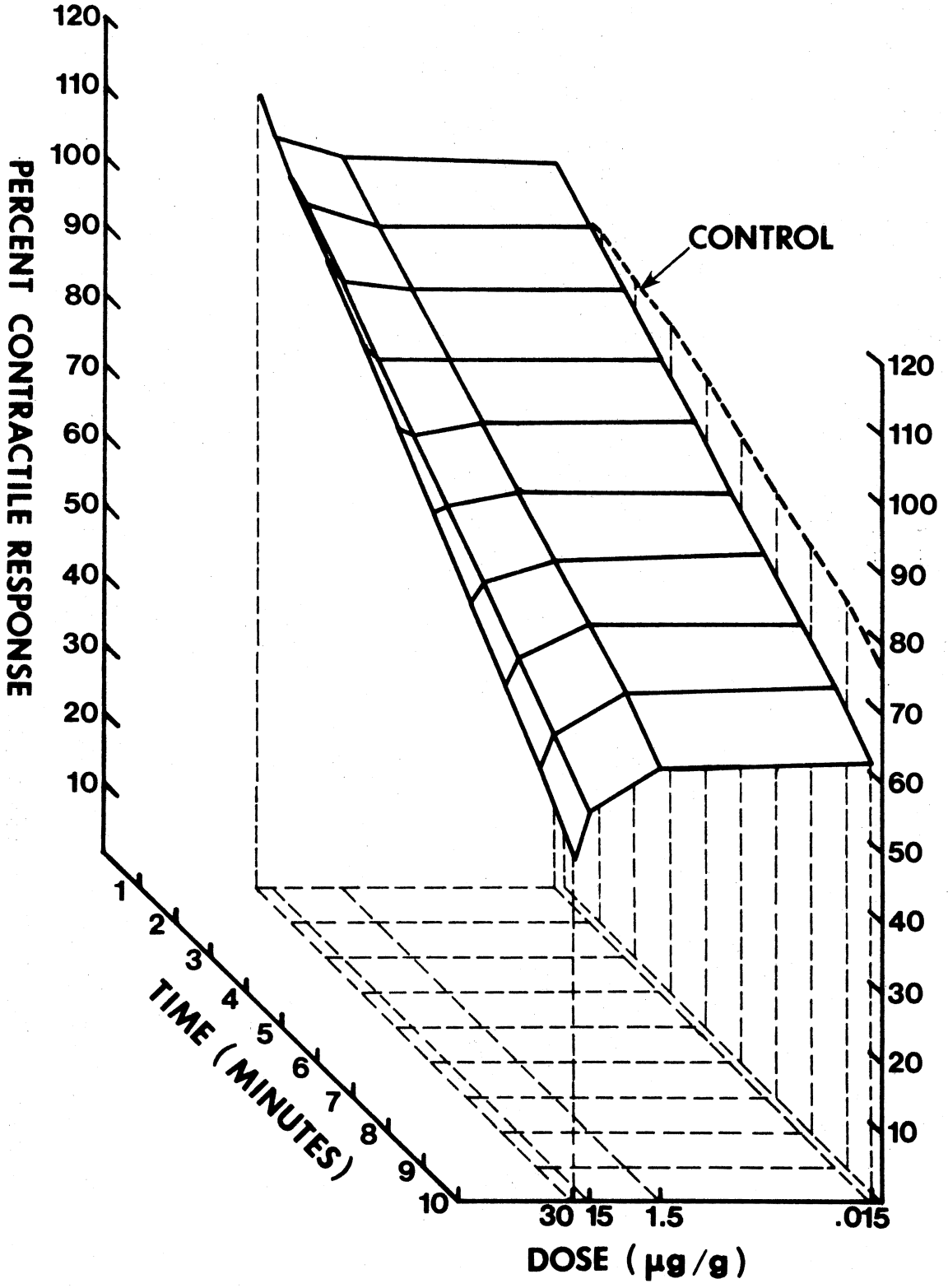

PERCENT CONTRACTILE RESPONSE
110
100
90
80
70
60
50
40
30
20
10
0
CONTROL
TIME (MINUTES)
1
2
3
4
5
6
7
8
9
10
300.
3.
.03
DOSE (µg/g)

Figure 3. Surface Response of Myotoxin a-Treated Muscles and Untreated Physiologic Control. All values plotted are means of the four replicates. Note all doses of myotoxin a produce a greater rate of decline of contractile response than the untreated muscles.


PERCENT CONTRACTILE RESPONSE
120
110
100
90
80
70
60
50
40
30
20
10
CONTROL
TIME (MINUTES)
1
2
3
4
5
6
7
8
9
10
DOSE (µg/g)
30
15
1.5
.015

X_1 = concentration of tested agent;

X_2 = time in minutes after addition of test agent; and

B_{0-5} = constants generated from raw data for each test agent.

The three generated surfaces were hyperboloids whose equations are:

Crude venom:

$$Y_{ij} = 1.0493 + 0.00000355\ X_1 - 0.0347\ X_2 + 0.000621\ X_2^2 - 0.00000017\ X_1X_2$$

Myotoxin a:

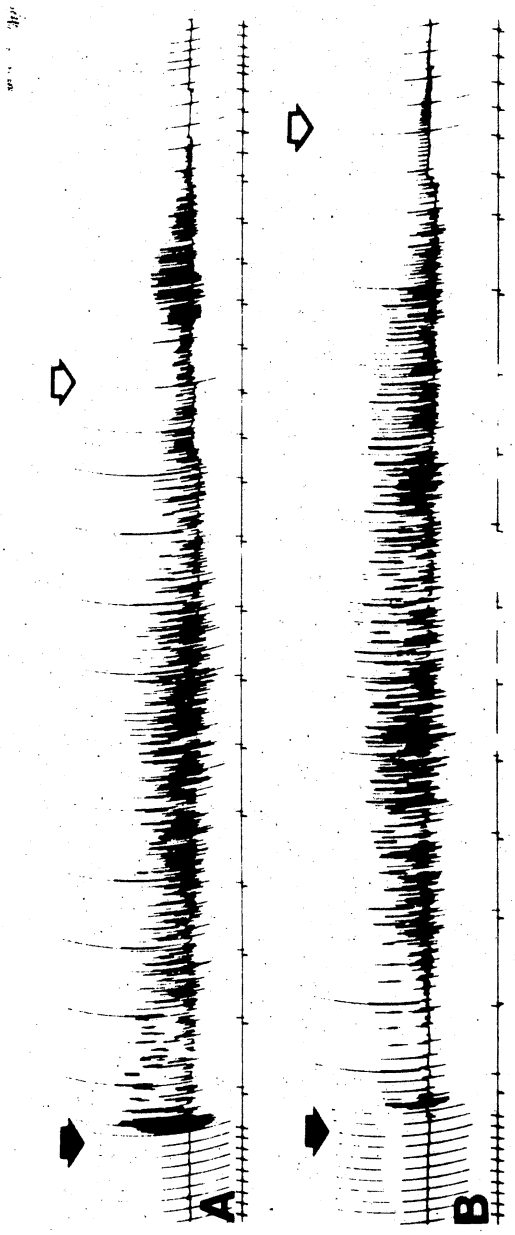
$$Y_{ij} = 1.0865 + 0.00000268\ X_1 - 0.04213\ X_2 - 0.000274\ X_2^2 - 0.00000088\ X_1X_2$$

Ouabain:

$$Y_{ij} = 1.0094 - 0.00000061\ X_1 - 0.015667\ X_2 - 0.002582\ X_2^2 - 0.0000001\ X_1X_2$$

Two other responses to the agents were observed during the physiologic experiments. The first of these was a spasm produced by the two highest doses (30.0 and 15.0 μg/g) of myotoxin a and by the highest dose (300.0 μg/g) of crude venom, as shown in Figure 4. These spasms began within 15 seconds after addition of the agent and continued up to 2 minutes. The second reaction was an enhancement of contractile response (in excess of 100%) produced by the same agents and doses as above which may be seen upon close observation of the surface response (Figures 1, 2, and 3). These enhancements appear for the first 1 to 2 minutes following addition of the agent doses specified above. Ouabain did not produce either of these responses at any of the doses tested.

Figure 4. Actual Physiograph Recording of the Contractile Response. Closed arrow indicates addition of the agent; open arrow indicates one minute after addition of the agent. A. Crude venom (300.0 µg/g). B. Myotoxin a (15.0 µg/g). Note that the spasms begin within 15 seconds after addition of agent and continue for approximately 1 minute. Each marker (lower scale) indicates 5 seconds. Maximum deflection (upper scale) is approximately 20 grams.


A
B

Measurements of pH and osmolarity of the bathing solutions indicated only slight variations following completion of the physiologic segment of the experiments. A decrease in pH of less than 0.3 pH units and an increase in osmolarity of less than 30 milliosmoles was observed to be the extreme limits of variation in any of the bathing solutions.

Morphologic Studies

Light Microscopy

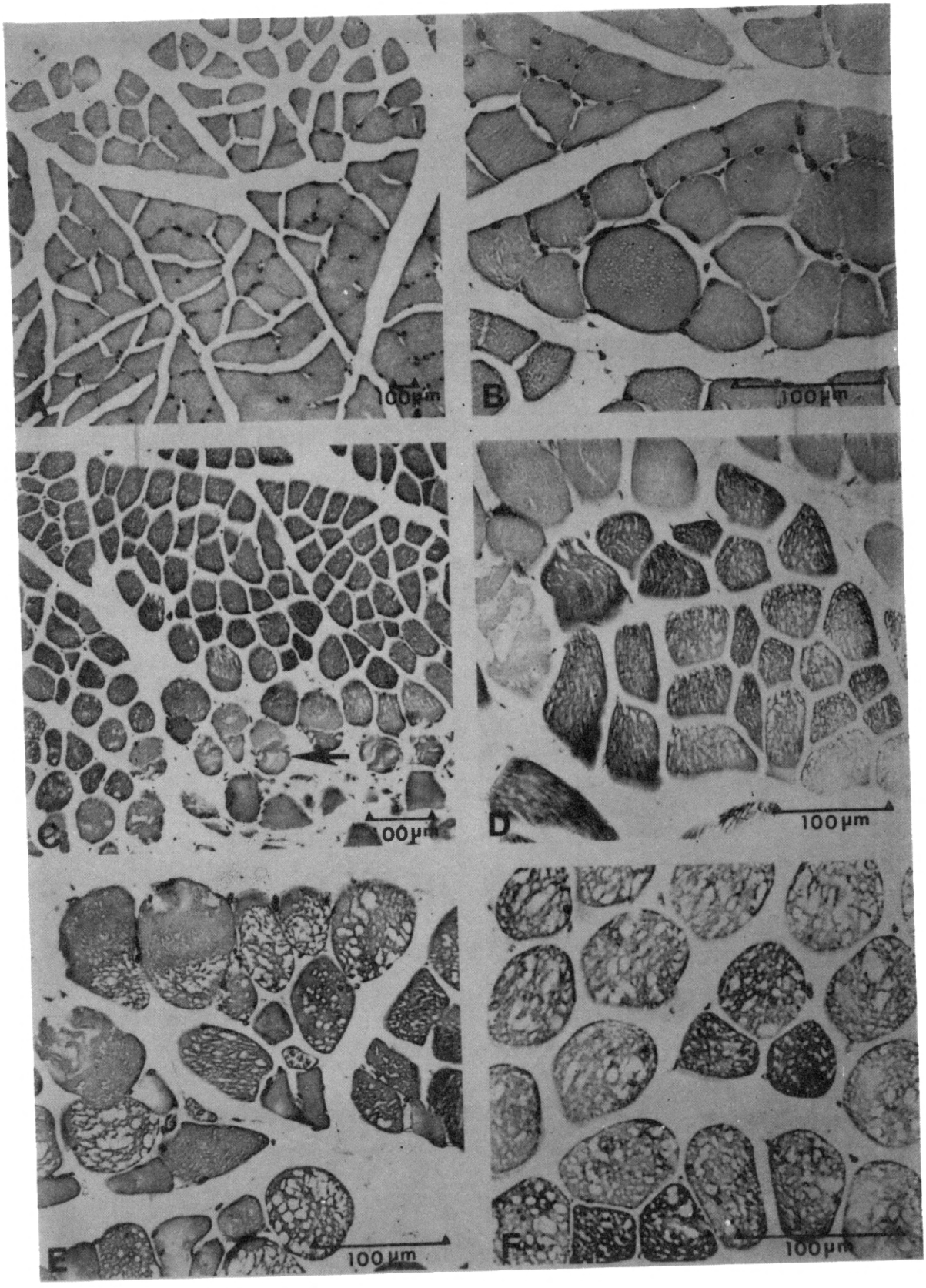
Samples obtained from the untreated physiologic controls after recording the muscle response showed minute vacuole formation along the periphery (Figure 5A). However, the interior region of the muscle preparation appeared normal and well-preserved for skeletal muscle.

All treatments used in this study produced visible effects on the exterior portions of the muscle preparation. The extent of detectable damage varied with dose, but no dose affected 100 percent of the muscle fibers in the preparation.

Examination of the low dose of ouabain revealed vacuole formation (Figure 5B). The sarcolemma of all cells appeared intact and muscle bundle configuration was normal. Several of the cells appeared swollen and rounded and contained large numbers of small vacuoles (Figure 5B), while others appeared unaffected by this treatment. Fewer vacuoles were observed in the muscle treated with higher doses (data not shown).

The muscles treated with crude venom contained cells with vacuoles and cells in various stages of degeneration at all doses tested in this study. At low doses (3.0 µg/g) damage to muscle cells ranged from slight vacuolation to a coagulated appearance of the normal sarcomere arrangement (Figure 5C). The highest dose used (300.0 µg/g) produced extensive

Figure 5. Light Micrographs of Muscles Taken Immediately After the Contraction Studies. A. Untreated physiologic control; note no gross abnormalities in muscle. B. Ouabain-treated sample (0.015 µg/g); note rounded cell which contains small vacuoles. C. Crude venom-treated sample (0.03 µg/g); note vacuolated cells; arrow indicates coagulation. D. Crude venom-treated sample (300.0 µg/g); note extensive vacuolation and coagulated cell. E. Myotoxin a-treated sample (0.015 µg/g); note similar appearance to muscle in Figure 5D. F. Myotoxin a-treated sample (30.0 µg/g); note extensive vacuolation and lack of coagulation.


A
100µm
B
100µm
C
100µm
D
100µm
E
100µm
F
100µm

vacuole formation and some fiber coagulation in the affected cells (Figure 5D). The number of affected cells increased with increases in dose, while the size and number of vacuoles within a cell was highly variable. Many cells appeared swollen and rounded, while most of the cells in the interior of the muscle preparation showed no effect of the treatment. Edema was present while no obvious hemorrhage was observed.

Myotoxin a induced muscle degeneration and vacuole formation at all doses tested. At the lowest dose (0.015 μg/g) the cells were seen in various degrees of degeneration ranging from no vacuoles to completely vacuolated to complete disruption of the normal striated appearance (Figure 5E). At the higher doses (15.0, 30.0 μg/g) the number of affected cells increased. These cells were swollen and contained vacuoles of various sizes (Figure 5F).

Electron Microscopy

The samples obtained from the soleus muscles taken immediately from the animal showed typical ultrastructural organization of skeletal muscle (Huxley, 1958; Pellegrino & Franzini-Armstrong, 1969). All cellular components appeared to be well-preserved and normal for mammalian skeletal muscle (Figure 6A).

Samples taken from the untreated physiologic controls showed slightly swollen sarcoplasmic reticulum (Figure 6B). However, the perinuclear space mitochondria, sarcolemma, and myofilaments appeared typical for skeletal muscles.

Electron microscopic examination of the ouabain-treated muscles confirmed that the vacuoles observed at the light microscopic level were swollen sarcoplasmic reticulum. Myeloid figures were present in some of

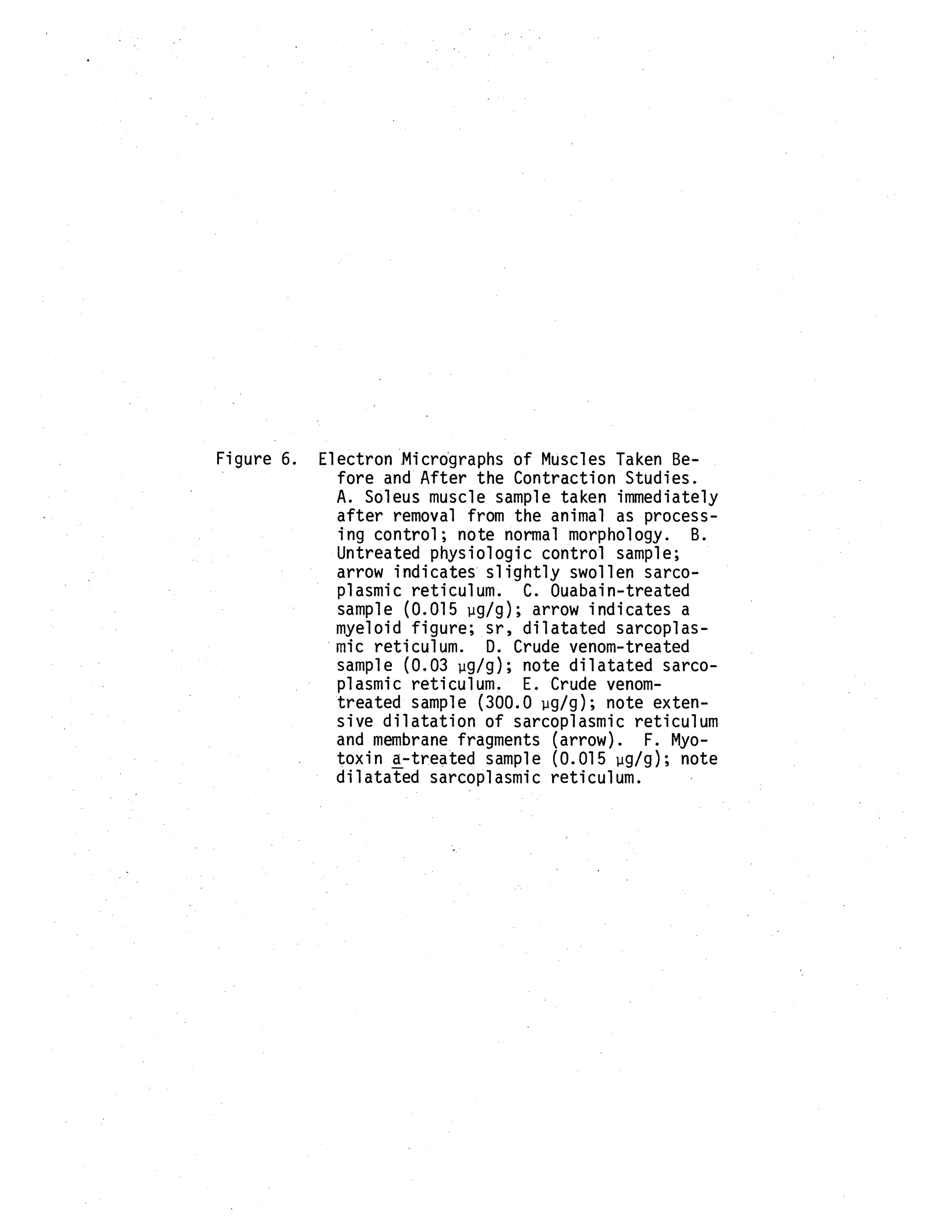
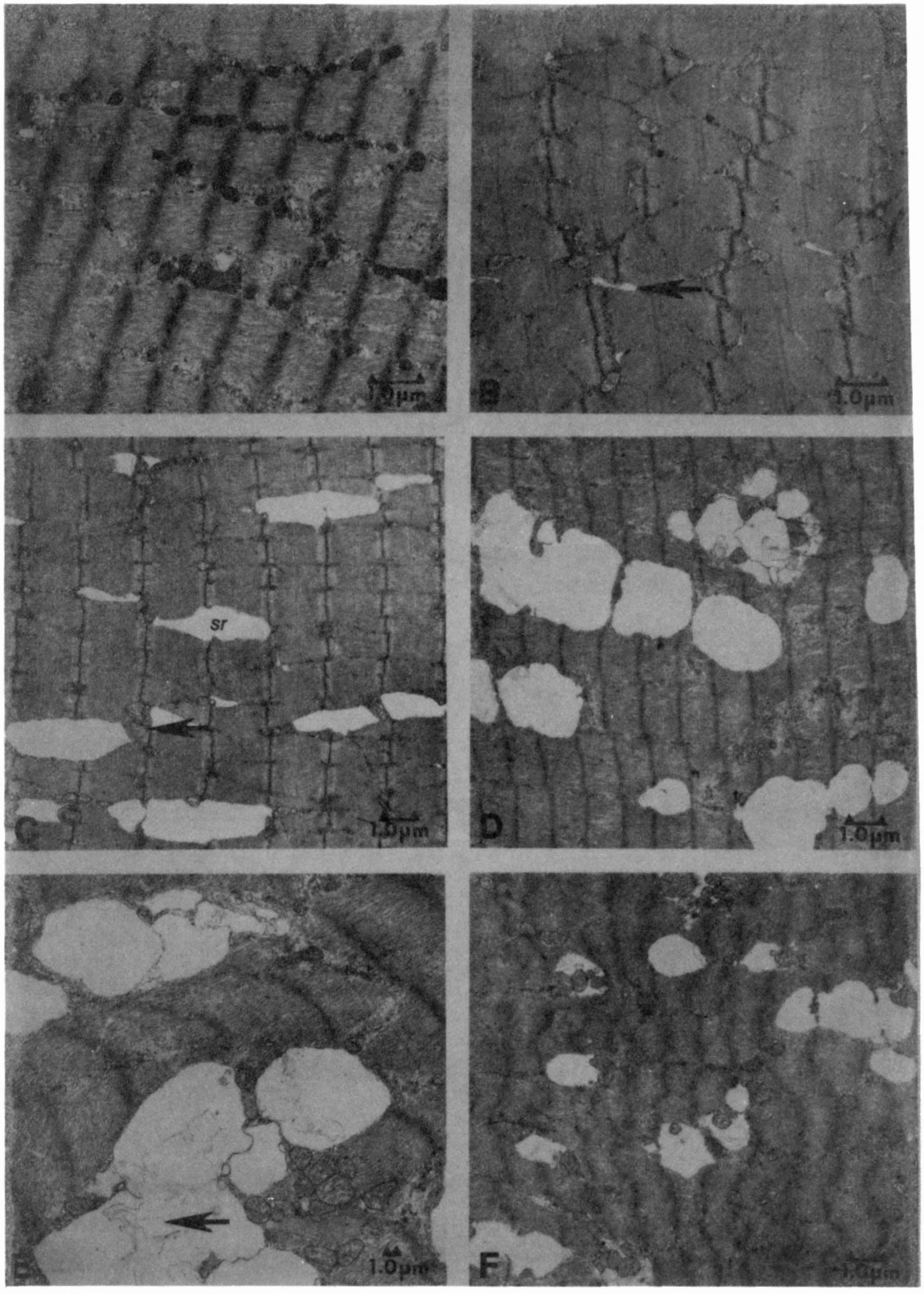


Figure 6. Electron Micrographs of Muscles Taken Before and After the Contraction Studies. A. Soleus muscle sample taken immediately after removal from the animal as processing control; note normal morphology. B. Untreated physiologic control sample; arrow indicates slightly swollen sarcoplasmic reticulum. C. Ouabain-treated sample (0.015 µg/g); arrow indicates a myeloid figure; sr, dilatated sarcoplasmic reticulum. D. Crude venom-treated sample (0.03 µg/g); note dilatated sarcoplasmic reticulum. E. Crude venom-treated sample (300.0 µg/g); note extensive dilatation of sarcoplasmic reticulum and membrane fragments (arrow). F. Myotoxin a-treated sample (0.015 µg/g); note dilatated sarcoplasmic reticulum.


1.0μm
B
1.0μm
sr
C
1.0μm
D
1.0μm
E
1.0μm
F
1.0μm

the affected cells (Figure 6C). Perinuclear spaces were also dilatated. Myofilaments, mitochondria, and nuclei appeared undisturbed by this agent.

The muscles treated with crude venom displayed extensive dilatation of the sarcoplasmic reticulum under electron microscopic examination (Figures 6D and 6E). The perinuclear spaces were also dilatated. In some cases these vacuoles contained fragments of membranes (Figure 6E). This agent had no apparent effect on the nuclei, mitochondria, or myofilaments in the cells in the initial stages of degeneration.

Myotoxin a produced swelling of the sarcoplasmic reticulum (Figure 6F) and perinuclear space. The mitochondria, nuclei, and myofilaments appeared unaffected in muscle cells in the initial stages of degeneration.

CHAPTER IV

DISCUSSION

The results indicate that these agents (ouabain, crude venom, and myotoxin a) do not have the same effect on the contractile response of skeletal muscle. Least squares analysis of the data showed a dose-dependent response within each agent at approximately the 95 percent confidence level (p of 0.0563). Statistical comparison among agents with respect to time and dose showed a highly significant difference in response (p of 0.0001), which indicated that each agent induced a different pattern of contractile response in respect to dose within each agent.

An indication of the reliability of the above values was obtained by least squares analysis of variance among replicates and agents. No significant difference among replicates (p of 0.1000) was indicated in this study. When the response data were grouped by agent alone, no significant difference (p of 0.3486) was indicated. This confirmed that the selected doses tested within each agent produced similar amounts of variances in contractile response. A comparison of the response surfaces of the test agents with the response of the untreated physiologic controls shows that all doses of agents tested produced a decline greater than that of the untreated muscles (see Figures 1, 2, and 3).

The spasms observed with addition of the previously mentioned doses of crude venom and myotoxin a (Figure 4) are another indication that these two agents do not act in the same manner as ouabain. Since the

crude venom contains approximately 18 percent myotoxin a (Cameron & Tu, 1977), it is possible that myotoxin a is responsible for these spasms in the crude venom treatment.

The initial ultrastructural changes induced by these three agents appear similar: dilatation of the sarcoplasmic reticulum. This study confirms previous morphologic studies of the three agents: ouabain (Ginn et al., 1968), crude venom (Stringer et al., 1971), and myotoxin a (Ownby et al., 1976). Since the sarcoplasmic reticulum appears to be the primary reservoir for water influx (Ginn et al., 1968), it can be surmised that these agents all somehow cause (either directly or indirectly) water influx into the cell.

Ouabain is known to inhibit the Na-K ATPase (Akera et al., 1970; Besch et al., 1969; Caldwell & Nash, 1977; Rhee et al., 1976) which leads to a (physiologic effect) decrease in contractile response in high doses. However, our study indicates that ouabain and myotoxin a do not give the same physiologic response. Thus, myotoxin a probably does not affect the Na-K ATPase system.

Myotoxin a could induce an increase in the permeability of the sarcolemma to calcium ions and subsequently to water. The spasms induced at high doses and measurable declines at low doses could both be explained by increased permeability of the muscle cell to calcium ions. In addition, the morphologic effects of influx of calcium ions would probably be similar to those induced by the influx of sodium ions, since water would flow into the cell in both instances and cause swelling of the sarcoplasmic reticulum. However, the physiologic effect would be very different since the skeletal muscle cell may respond to increased calcium ion

concentration by enhanced contractile response and spasms. Increased sodium ion concentration would probably not produce either of these effects.

REFERENCES

Akera, T., Larsen, F. S., and Brody, T. M.: Correlation of cardiac sodium- and potassium-activated adenosine triphosphatase activity with ouabain-induced inotropic stimulation. J. Pharmacol. Exp. Ther. 173:145-151, 1970.

Barr, A. J., and Goodnight, J. H.: Statistical Analysis Systems. Dept. of Statistics, North Carolina State University, 1972.

Besch, H. R., Allen, J. C., Glick, G., and Schwartz, A.: Correlation between the inotropic action of ouabain and its effects on subcellular enzyme systems from canine myocardium. J. Pharmacol. Exp. Ther. 171:1-12, 1969.

Caldwell, R. W., and Nash, C. B.: Comparison of the effects of amino-sugar cardiac glycosides with ouabain and digoxin on Na^+, K^+--adenosine triphosphatase and cardiac contractile force. J. Pharmacol. Exp. Ther. 204:141-148, 1977.

Cameron, D., and Tu, A.: Characterization of myotoxin a from the venom of prairie rattlesnake (Crotalus viridis viridis). Biochem. 16: 2546-2553, 1977.

Ginn, F. L., Shelburne, V. D., and Trump, B. F.: Disorders of cell volume regulation. Am. J. Path. 53:1041-1058, 1968.

Homma, M., and Tu, A. T.: Antivenom for the treatment of local tissue damage due to envenomation by Southeast Asian snakes: Ineffectiveness in the prevention of local tissue damage in mice after envenomation. Am. J. Trop. Med. Hyg. 19:880-884, 1970.

Huxley, H. E.: The contraction of muscle. Sci. Am. 199:67-82, 1958.

Lindower, J., and Marks, B.: Ultrastructural changes in guinea-pig myocardium after acute ouabain treatment. J. Pharmacol. Exp. Ther. 202:76-88, 1977.

Luft, J. H.: Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:409-414, 1961.

Lüllmann, H., and Holland, W.: Influence of ouabain on an exchangable calcium fraction, contractile force, and resting tension of guinea-pig atria. J. Pharmacol. Exp. Ther. 137:186-192, 1962.

Minton, S. A., Jr.: Polyvalent antivenom in the treatment of experimental snake venom poisoning. Am. J. Trop. Med. Hyg. 3:1077-1082, 1954.

Ownby, C. L., Cameron, D., and Tu, A. T.: Isolation of myotoxic component from rattlesnake (Crotalus viridis viridis) venom. Am. J. Path. 85:149-159, 1976.

Pellegrino, C., and Franzini-Armstrong, C.: Recent contributions of electron microscopy to the study of normal and pathological muscle. Int. Rev. Exp. Pathol. 7:139-226, 1969.

Rhee, H. M., Dutta, S., and Marks, B. H.: Cardiac NaK ATPase activity during positive inotropic and toxic actions of ouabain. European J. of Pharmacol. 37:141-153, 1976.

Richardson, K. C., Jarett, L., and Finke, E. H.: Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Techonol. 35:313-323, 1960.

Stahnke, H. L.: The treatment of venomous bites and stings. Tempe, Ariz. Bureau Publications of Arizona State University, 1966.

Steel, R. G. D., and Torrie, J. H.: Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York, 1960.

Stringer, J. M., Kainer, R. A., and Tu, A. T.: Myonecrosis induced by rattlesnake venom: An electron microscopic study. Am. J. Path. 67: 127-140, 1972.

Venable, J. H., and Coggeshall, R.: A simplified lead citrate stain for use in electron microscopy. J. Cell. Biol. 25:407-408, 1965.

Vick, J. A., Cinchta, H. P., and Manthei, J. H.: Pathophysiological studies of ten snake venoms. In: Animal Toxins. F. E. Russell and P. R. Saunders, Pergamon Press, New York, 1967, pp. 269-282.

VITA

James P. Knight

Candidate for the Degree of

Master of Science

Thesis: A COMPARATIVE STUDY ON THE EFFECTS OF OUABAIN, RATTLESNAKE (CROTALUS VIRIDIS VIRIDIS) VENOM, AND MYOTOXIN a ON THE CONTRACTILE RESPONSE AND ULTRASTRUCTURE OF RAT SKELETAL MUSCLE

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Tulsa, Oklahoma, May 14, 1951, the son of Alfred B. Knight and Patricia Jean Dunn.

Education: Graduated from Cascia Hall High School in May, 1969; attended United States Marine Corps electronic schools in California from April, 1970, to March, 1971, and was honorably discharged in December, 1972; received the Bachelor of Science degree from Oklahoma State University in May, 1976, with a major in Zoology; completed requirements for the Master of Science degree at Oklahoma State University in July, 1978.

Professional Experience: Teaching assistant, September, 1976, to December, 1976; laboratory assistant, January, 1977, to May, 1977; research assistant, July, 1977, to June, 1978.