THE EFFECTS OF SPECIFIC EXERCISE REGIMENS ON THE MORPHOLOGY OF THE LEFT SOLEUS NERVE OF THE MALE ALBINO RAT

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ABSTRACT

THE EFFECTS OF SPECIFIC EXERCISE REGIMENS ON THE MORPHOLOGY OF THE LEFT SOLEUS NERVE OF THE MALE ALBINO RAT

By

Roland R. Roy

The purpose of this investigation was to determine the effects of four durations of five specific levels of physical activity on the population of myelinated fibers in the soleus nerve of the normal, male albino rat.

Eighty animals were brought into the laboratory and assigned randomly to seven treatment groups. Due to difficulties in preparation techniques, only five treatments were utilized in this study: sedentary control (CON); short-duration, high intensity running (SHT); long-duration, low intensity running (LON); electrical stimulus control (ESC); and endurance swimming (SWM). Animals were provided with food and water *ad libitum*. Treatments were administered Monday through Friday under controlled environmental conditions.

Animals from each group were sacrificed at zero weeks and then at four, eight and twelve weeks after the onset of training. The healthiest and best trained animals were selected for sacrifice. The final sample size consisted of 32 animals.

The left soleus nerve was surgically removed from sodium pentobarbital anesthetized animals. Upon removal, the nerve was stretched

to its physiological length on an absorbant cardboard square and placed into Bouin's fixative solution. The myelin sheath then was stained by immersion of the nerve section into a .5% solution osmium tetroxide, embedded in paraffin blocks and sectioned at 7 micra.

Visual images of the entire transverse section of the nerve were projected onto drawing paper with a Bausch and Lomb microprojector at a magnification of X1,133. Myelin, axon, and total areas of each myelinated fiber were measured by polar planimetry. Total fiber counts were recorded during the same process.

Percent frequency graphs were constructed for myelin, axon and total areas across durations and treatments. Visual inspection of the plots revealed patterns of specificity in the data. SHT and ESC animals tended to have a larger percentage of small fibers than did the CON, LON and SWM groups. The graphs of all experimental groups were notably different from those of the CON group. Chi square analyses run on specific pooled groups substantiated the observed trends. Total fiber counts appeared to be decreased by all training regimens across durations.

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Roland R. Roy

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Dedication

To my family - for making all this worthwhile

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LIST OF ABBREVIATIONS

ACT	Analysis of contingency tables
CDS	Cumulative duration shock. The duration of shock (seconds) received by experimental animals (SHT, LON) and control animal (ESC) during all work periods of all bouts of a given training period.
CON	Sedentary control
CRW	Controlled running wheel
EST	Expected swim time (minutes)
LON	Long-duration, low-speed, endurance running exercise (long CRW program)
PER	Percent expected revolutions PER = 100 TRR/TER
PET	Percent expected swim time PET = 100 STC/EST
PSF	Percent shock free time PSF = 100-(100 CDS/TWT)
SHT	Short-duration, high-speed, endurance running exercise (short CRW program)
STC	Swim time completed (minutes)
SWM	Long duration swimming exercise (in individual tanks)
TER	Total expected revolutions that an experimental animal (SHT, LON) would run during all work periods of all bouts of a given training period, if he would run at the prescribed speed.
TR	Trained animals. A pooled distribution of SHT, LON, ESC and SWM data.
TRR	Total number of revolutions run by the experimental animal during all work periods of all bouts of a given training period (for animals in the SHT and LON groups).
TWT	Total work time (seconds) during all work periods of all bouts for a given training period.

CHAPTER I

THE PROBLEM

Investigations reporting the effects of exercise or functional overload on the morphology of peripheral nerves have been extremely diverse in their findings. Experimental results have ranged from marked hypertrophy and hyperplasia of the nerve fiber population (85, 25) to atrophy and reduction in total fiber number (5). However, the evidence presented has often been confounding and impossible to interpret because in many cases the training regimens used have not been well defined. In addition, many of the studies produced increased or decreased levels of activity through various surgical procedures, e.g. tenotomy and tenectomy. In many cases, the direct effects of these procedures on the obtained results was left undetermined.

Recent evidence has suggested that exercise consists of a continuum of activity levels each eliciting a specific response within the organism. This information has implied that the effects of a long-distance, endurance running program may be quite different from the effects of a short-distance, high intensity running program. Thus, well defined and closely controlled exercise regimens must be utilized to determine these specific effects of exercise.

Only one study has attempted to investigate the specificity of exercise effects on the morphology of the peripheral nervous system (PNS) (82). Acute, chronic and limited muscular activity were the

three experimental treatments investigated. Only limited muscular activity resulted in significant changes in the nerve-fiber population.

Statement of the Problem

The purpose of this investigation was to study the chronic effects of four durations of five well-defined activity levels on selected morphological characteristics of the soleus nerve of the male, albino rat. Specifically, the cross-sectional areas of the axis cylinder and its associated myelin sheath were measured for each nerve fiber in the population. In addition, the total number of myelinated fibers in each nerve was recorded.

Significance of the Problem

The nervous system is the functional unit in the human body which activates, controls and coordinates most of the other systems. Along with the endocrine system, it provides the control mechanism for all bodily functions. Consequently, there are many implications in finding morphological changes within the nervous system.

An increase in the myelin content of the individual nerve fibers could be one possible explanation for increased speed of movement in some well-trained performers. Similarly, an increase in the number of myelinated fibers may account for the smooth coordinated and discrete movements found in highly skilled gymnasts and dancers. However, such theories have yet to be substantiated.

The importance of the present study rests primarily in the depiction of the specific effects of well-defined, reproducible exercise regimens on the gross morphological characteristics of a peripheral nerve.

Limitations of the Study

1. The results of this study cannot be applied directly to the human being.

2. The durations of the treatment periods were somewhat arbitrary and therefore not necessarily optimal for obtaining significant results.

3. The training programs were selective and therefore not necessarily representative of other discrete types and intensities of activity.

4. The measuring techniques employed restricted the accuracy of the recordings at the smaller and larger ends of the fiber spectrum.

5. The total sample size limited the power of the statistical analyses.

CHAPTER II

REVIEW OF RELATED LITERATURE

The purpose of this study was to determine the effects of five specific exercise regimens on the morphology of the left soleus nerve of adult male albino rats. The parameters investigated were the myelin sheath area, the axonal area and the total number of myelinated fibers in the nerve trunk.

The following review of related literature will be divided into three sections: (a) To gain perspective of the problem, the first section will be devoted to the current ideas and theories on nerve growth. The variables affecting axonal size and development will be considered. The mechanism and role of axoplasmic transport as it relates to cell body-axon-terminal connection interrelationships also will be briefly discussed. (b) Axon-myelin relationships, with emphasis on the controlling mechanisms of myelin-sheath thickness, will be presented in the following section. (c) Finally, the effects of various levels of activity on the gross morphological characteristics of the peripheral nerve and, in some cases, its associated alpha motor neuron will be reviewed.

Nerve Fiber Growth

Nerve-fiber growth involves two distinct processes, elongation and enlargement. Although concurrent during the development of the animal or in the early stages of regeneration, these two processes are

quite separate in the adult animal. For clarity, each process will be discussed separately in the following review.

Elongation begins as a protoplasmic extension from the nerve cell body (43, 75). The advance of the protoplasm continues until the free tip of the fiber attaches permanently to a peripheral connection. Continued lengthening of the fiber is then accomplished passively in response to the tug of the terminal organ in a process called "towing" (90). Elongation stops when the animal has reached full maturity.

Enlargement, or true growth, is the result of the production of new protoplasm at a rate which exceeds the elongation process and increases the width of the axon. The production of new protoplasm has been shown to occur solely at the base of the fiber in the nucleated part of the cell body (46, 80, 88, 89). In the mature animal, the perikaryon reproduces new axoplasm at a rate determined by the constitutional properties of the cell and by various external conditions. The nerve fiber is the outlet into which the new axoplasm is siphoned by an axomotile mechanism. The substances move down the nerve processes in response to active constriction and distension of the axis cylinder (35, 74, 87) and, in some cases, in response to contractions in the cell body itself (52). The width of the fiber is normally adjusted so that an unobstructed passage of the axoplasm at a commensurate rate is permitted. The moving axoplasm is then consumed in the metabolic activity of the fiber (93).

In the classical series of nerve regeneration and constriction studies performed by Weiss and Hiscoe in 1948 (93), this mechanism of axonal transport was unequivocally demonstrated. The evidence presented was in terms of the permanent size deficit at the distal side of a constriction imposed on a nerve fiber and the "damming" or buildup of

axoplasm at the proximal side. The authors ultimately concluded that the caliber of a nerve fiber was essentially determined by two factors, the amount of synthesis of new axoplasm in the cell body and the rate of its centrifugal movement. Later evidence of axonal transport came from radioautographic and biochemical studies on the delivery into the axon of isotopes incorporated into substances of the cell body (17, 18, 79) and from lapsed-time cinematography of the living nerve fiber (94). The work accomplished in the area of axonal transport has been extensively reviewed by Lubinska (53) and by Barondes and Samson (7).

A third factor was introduced when the influences of peripheral factors upon the process of nerve growth were investigated. Several regenerative studies (3, 4, 24, 28, 67, 68, 95, 96) demonstrated that the most powerful peripheral influence is the contact which is made with the end organs. The basic result of the lack of peripheral connections is the atrophy of the nerve fibers (3, 68, 96) and the related cell body (11). Connections with functionally inappropriate organs proved to be inadequate substitutes for normal connections (4, 72, 96). However, it also was shown that nerve growth is not dependent upon a normal contraction of muscle against resistance. Some muscle substance is essential for the promotion of growth, but the actual amount does not seem to be critical (28). The maturation is much greater in a nerve connected with a tenotomized muscle than in one connected with no periphery at all (4).

The manner in which the terminal connections influence the nerve cell body and axon is still under investigation. The chromatolytic reaction of the cell after peripheral severance of its axon indicates that the perikaryon has received information about the peripheral

disturbance (11, 96). A transport system from the periphery to the cell body has been suggested on the bases of investigations in which axons were transected and changes studied in the perikaryon (57) and in radioactive studies of isolated CNS-nerve trunk-muscle preparations (50). In addition, direct observations of individual nerve fibers in tissue culture and in vivo have shown bidirectional movements of granules in the axons and centripetal migration of particles taken up by pinocytosis at the axon tips (53). This evidence suggests the existence of a continual migration of neuronal cytoplasm from the cell body to the nerve endings and from the nerve endings back to the cell body.

Axon-Myelin Relationships

Light microscopic investigations indicate that the myelin sheath thickness is in direct proportion to the caliber of the axis cylinder (19, 69, 101). These findings have recently been substantiated by electron microscopic work (30, 31, 32, 33, 54, 55). A critical diameter of the axon for the initiation of myelin production has been shown to exist, and it ranges from one to two micra in the cat, rat and cow. Myelination appears to be initiated in this diameter range regardless of the function of the fiber, or the age, size or species of the animal (19, 55).

Martinez and Friede (54) have demonstrated that the dimensions of the axon control the myelin forming activity of the Schwann cells. Axonal growth was explained by studying the changes in the perikarya of the nerve cells. An increase in the volume of axoplasm was found to be almost equal to, and to develop parallel with, an increase in the volume of cytoplasm in the lower motor neurons. Coupled with the

findings that the formation of myelin lamellae occurs in proportion to changes in axonal circumference (32, 33), investigators have postulated the following mechanism of axon-myelin interdependence: (a) the primary changes occur in the cell body of the neuron which produces and controls a correlated growth of the perikaryon and its axon. (b) The resulting increase in axonal caliber controls the myelin formation by the Schwann cell. In addition, a model of a mechanism controlling sheath growth itself has been postulated (30).

Effects of Exercise and Functional Overload on Nerve Growth

The effect of increased or decreased activity on the nerve cell and its processes is very controversial at this time. Some work has been done in this area but the results are inconclusive.

One of the earliest exercise studies was conducted by Dolley in 1913 (15). Dolley attempted to determine the effect of exhaustive functional activity, as well as depression of such activity, on nerve cells. The result in both cases was a complete cessation of function. In effect, the study demonstrated that going to either extreme of the activity continuum was detrimental to the nerve cell.

Later studies have supported the view held by Dolley. Running guinea pigs to exhaustion has been shown to deplete the protein stores in the nerve cells (46) and to decrease the mean diameter and total number of the nerve fibers (5). A significant decrease in protein nitrogen following motor activity also was demonstrated in rats swum to exhaustion (47).

Conversely, other investigations have shown a beneficial effect of increased activity on the nerve cell and its processes. Agduhr (2)

noted both an increase in the mean fiber number and the mean fiber diameter in the dorsal and ventral nerve root fibers of mice exercised in rotating cages. Edds (25) conducted a study designed to show the effects on the nerve fibers of functionally overloaded muscles. Comparative measurements were made on the number and caliber of all myelinated fibers in nerves to the soleus muscle of adult rats which had undergone functional hypertrophy following the denervation of some of their synergists. The results showed an increase in the number of nerve fibers in experimental as compared to control animals. In addition, the myelinated fibers increased in size, and the distribution of fibers shifted from all size classes to larger size classes.

Experiments in partially deneurotized nerves yielded similar results (24). The plurisegmental, long thoracic nerve of the rat was deneurotized by removing one of its contributory spinal nerves. The residual axons in such a nerve produce intramuscular, collateral sprouts which reinnervate many of the denervated muscle fibers (23) and thus functionally overload the nerve. Hypertrophy of the residual axons was reflected in shifts of all fiber size classes to larger size classes.

Functional overload of the medial belly of the gastrocnemius, induced by denervation of synergists in the rabbit, also has been shown to increase both the number and size of myelinated nerve fibers (85).

Levels of inactivity have been simulated in various manners. Tenotomy, the surgical removal of a tendon, has been used extensively to induce levels of depressed muscular activity (21, 44). The influence of this procedure on the diameter and number of nerve fibers is uncertain. Aitken et al. (4) reported an increase in total fiber number and no change in the mean fiber diameter of the myelinated fibers to the

tenotomized gastrocnemius muscle of the rabbit. Evans and Vizoso (28), on the other hand, found decreases in the numbers of fibers with diameters larger than 9 micra in the same nerve. Thus, the results are inconclusive.

Recently Tomanek and Tipton (82) undertook an experiment to reinvestigate the influence of increased and decreased muscular activity on the diameters and number of myelinated fibers comprising the medial gastrocnemius nerve of the rat. Acute exercise (a single bout), chronic exercise (training on a motor driven treadmill five to six times per week for 8 weeks at various times and speeds), and tenectomy (limited muscular activity brought about by excision of the tendocalcaneous) were the treatments investigated. The only significant effects found were with tenectomy. The tenectomized animals showed statistically significant decreases in both nerve-fiber diameter and number. The exercise programs did not produce any significant changes.

CHAPTER III

MATERIALS AND METHODS

Sample

As subjects for several concurrent studies, eighty normal, 72-day-old, male albino rats (Sprague-Dawley strain)[1] were brought into the laboratory in two shipments. Each animal was randomly assigned to one of seven treatment groups and then allowed 12 days to adjust to the laboratory before treatments began.

This study involved only five of the treatment groups since the investigator was not able to collect data from animals in two of the groups. Application of selection criteria, to be discussed later, and difficulties in staining techniques resulted in the final sample consisting of 32 animals (Table 1).

Table 1. Final cell frequencies by treatment and duration

Treatment	Duration			
	0-wk	4-wk	8-wk	12-wk
CON	5	2	2	2
SHT		2	2	2
LON		2	2	2
ESC		2	2	1
SWM		1	1	2

[1]Obtained from Hormone Assay Laboratory, Chicago, Illinois.

Treatment Groups

The five treatment groups used in this study were as follows:

Control (CON)

These animals received no special treatment and were housed in individual sedentary cages (24 cm. long by 18 cm. wide by 18 cm. tall) during both the adjustment and the treatment periods.

Short (SHT)

The animals assigned to the short group were housed in individual voluntary cages (sedentary cages with access to a freely revolving activity wheel) during the adjustment period and in individual sedentary cages during the treatment period. These animals were subjected to a short-duration, high-intensity program of interval training in individual controlled-running-wheels for small animals (CRW) (98). The intensity of the training program was progressive in nature. At the end of thirty-seven days of training, the animals were expected to complete eight bouts of exercise with 2.5 minutes of inactivity between bouts. Each bout consisted of six repetitions of 10 seconds of work alternated with 40 seconds of rest. The required speed was a relatively fast 5.5 ft./sec. which would be comparable to that used in an anaerobic event in the human, i.e. the 100-yard dash.

Long (LON)

The animals in the long group were housed under the same conditions as the short group. These animals were subjected to a long-duration, low-intensity endurance program of interval training in individual CRW. The duration of the training program was progressively increased. At the end of thirty-seven days of training, the animals were expected to

complete four 12.5-minute bouts of exercise with 2.5 minutes of inactivity between bouts. Each bout consisted of one repetition of 12.5 minutes of continuous running. The required speed was a relatively slow 2.0 ft./sec. which would be comparable to that used in an aerobic event in the human, i.e. the mile run.

Electrical Stimulus Control (ESC)

These animals were housed in individual voluntary activity cages during the adjustment period and in individual sedentary cages during the treatment period. The group consisted of animals which were permanently paired with the animals in the SHT group. During each training period for the SHT group, the ESC animals were placed in adjacent stimulus control cages (21.5 cm. long by 14 cm. wide by 10.5 cm. tall) having grid floors electrically comparable to those of the CRW. Each ESC animal was thus exposed to the same total light and electrical shock stimuli as its paired counterpart.

Swim (SWM)

These animals were housed in individual voluntary activity cages during the adjustment period and in individual sedentary cages during the treatment period. The animals were swum in individual cylindrical tanks (28 cm. by 76 cm.) with a water temperature of 28°-32° C. The swimming program was progressive in nature such that on the last four days of the eighth week of training, each animal was expected to swim continuously for one hour with an attached tail weight equal to 3% of its body weight.

Duration Groups

Animals were sacrificed at zero, four, eight and twelve weeks after the initiation of the treatments. The training requirement for each treatment group increased progressively from zero to eight weeks. Twelve-week animals followed the same program from day 37 to day 60 (Appendix A). This procedure was followed in an attempt to show the patterns of change associated with chronic exercise programs.

Treatment Procedures

The treatments began after a 12-day adjustment period when all animals were 85 days old. Those animals selected as zero-week controls were sacrificed on the first day of the treatment period. All other animals began their individual treatment programs ranging from four weeks to twelve weeks in duration.

The SHT and LON exercise groups and one of the control groups (ESC) received treatment in the CRW apparatus that has been described as:

> "...a unique animal-powered wheel which is capable of inducing small laboratory animals to participate in highly specific programs of controlled reproducible exercise." (98)

Each animal was placed in an individually braked running wheel (CRW) and induced to run in response to a controlled, low-intensity shock current applied through the grid running surface of the wheel. A light stimulus preceeded the electrical stimulus so that the animals could avoid the electric shock by responding to the light. Most animals were conditioned to react to the light stimulus in a short period of time.

Each running period was initiated by releasing the brake and simultaneously turning on a light above the wheel. The light remained on for a predetermined amount of time, the acceleration time. Animals not attaining the prescribed speed by the end of the acceleration time were electrically stimulated through the grid apparatus. For those animals attaining the desired running speed, the light was turned off and no electric current passed through the bars. Animals running slower than the specified speed had the light and shock sequence repeated. During the work periods, the wheel was free to turn; while during the rest periods, the wheel was automatically braked to prevent spontaneous activity. A typical running program consisted of alternate work and rest periods.

Total revolutions run (TRR) and cumulative duration of shock (CDS) were recorded from a result unit attached to each CRW after every training period for the SHT and LON groups. The ESC animals used SHT values. Percent expected revolutions (PER) and percent shock free time (PSF) were calculated by comparing the recorded values to the programmed values for total expected revolutions (TER) and total work time (TWT).

The animals in the SWM group were swum in individual cylindrical tanks. Tail weights were calculated using body weight before treatment from the previous Friday and attached to the tips of the tails by means of miniature plastic clothespins. Expected swim times (EST) were progressively increased. If an animal was unable to complete the EST, the animal was removed from the tank and his swim time completed (STC) was recorded. The STC and EST were then used to calculate the percent expected swim time (PET).

The exercise and control (ESC) treatments were performed once a day, between 12:30 p.m. and 5:30 p.m., Monday thru Friday, in the Human Energy Research Laboratory, Michigan State University, East Lansing, Michigan. Body weights for SHT, ESC and LON animals were taken before and after each treatment period. Swim group animals were weighed only before their daily treatment.

Animal Care

Each animal was housed in a cage which was steam-cleaned every two weeks. The animals received food (blocks)[1] and water *ad libitum*. The ambient temperature in both the animal quarters and the treatment room was maintained between 70° and 72° F., and the relative humidity was kept between 40 and 60 percent. Standard procedures for CRW cleaning and maintenance were observed.

The animals were exposed to an automatically controlled daily sequence of twelve hours of light followed by twelve hours without light. Since the rat is normally a nocturnal animal, the light sequence was established so that the lights were kept off between 1:00 p.m. and 1:00 a.m. and turned on between 1:00 a.m. and 1:00 p.m. This lighting pattern reversed the normal day-night schedule for the animals so that they were trained during the active phase of their diurnal cycle.

Sacrifice Procedures

Six bimonthly sacrifices of seven animals of the same treatment duration were conducted between June 7 and August 16, 1971. Two additional sacrifices on November 22, 1971 and December 6, 1971 of

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[1]Wayne Laboratory Blox, Allied Mills, Inc., Chicago, Illinois.

eight animals each were performed involving only zero-week animals.

All animals were sacrificed on Monday following their last exercise period on the previous Friday. Approximately seventy-two hours elapsed between the last exercise period and sacrifice. Fifty-eight rats were sacrificed in total.

Animals were selected for sacrifice on the basis of their health and their performance during the treatment period. Only those animals subjectively determined to be in good health were picked for sacrifice. In addition, for CRW animals, only those rats completing at least 75 percent expected revolutions (PER) and maintaining no less than 75 percent shock free time (PSF) met the predetermined criteria for selection. A mean percent expected swim time (PET) of approximately 100 percent was used as the basic criteria for selecting animals from the SWM group.

Each animal was weighed and then sacrificed under anesthesia by an intraperitoneal injection of 4 mg./100 g. body weight of 6.48 percent sodium pentobarbital solution.[1] The left hindlimb was skinned and the superficial posterior crural muscles were exposed by reflecting the overlying tissue. The left triceps sural group (gastrocnemius and soleus) and plantaris were identified and dissected as a unit to their common attachment at the Achilles tendon. The soleus tendon was isolated and clamped with a hemostat. The tendon then was cut distal to the hemostat and the soleus was gently dissected towards its proximal attachment on the posterior femur. All fascia and connective tissue was cleared away with a blunt probe. The soleus nerve was identified in the proximal third of the muscle and ligated at both ends with fine white

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[1]From Jensen-Salsberg Laboratories, Division of Richardson-Merrell, Inc., Kansas City, Missouri.

thread. The nerve was cut as close to the muscle as possible so that the largest possible section was extricated.

Upon removal, the nerve section was placed on a precut piece of dry, absorbant cardboard and gently stretched to approximate its physiological length. All extraneous fascia and connective tissue were dissected from around the nerve sheath. A razor blade was used to cut through the nerve and to remove the pieces of string at either end. The remaining section adhered to the cardboard and maintained the desired tension. Each cardboard and nerve were immediately submerged in separate jars of Bouin's solution.

Preparation and Staining Techniques

After a one-to-eight-day period, the nerve sections were removed from the Bouin's solution, put through two one-half hour washings in distilled water and placed in a .5% solution osmium tetroxide for staining of the myelin sheath. After four hours, the sections were removed from the osmic acid solution and placed in a plastic container with running tap water for 24 hours. The following day, the nerve section was dehydrated and infiltrated with paraffin in the manner described by Adams (1). Within two hours after the last paraffin exchange, the nerve tissue was embedded in standard paraffin blocks and allowed to cool overnight.

Sectioning took place within one week. The paraffin sections were cut 7 micra thick on a sliding microtome placed in a water bath regulated at 30°-33° C., mounted on glass slides and air dried. The slides then were placed in a 37° C. oven for incubation for a least 24 hours.

To remove the excess paraffin, the incubated slides were totally immersed in xylene for approximately 20-30 minutes. The sections then were covered with permount, mounted and allowed to dry.

Measurement and Counting Techniques

Visual images of the entire transverse section of the nerve were projected onto drawing paper with a Bausch and Lomb microprojector at a magnification of X1,133. The outline of each myelinated fiber was traced with a sharp pencil. For each fiber, both the inside area, corresponding to the axon area, and the outside area, corresponding to the axon area plus the myelin area, were measured using a No. 620005 compensating polar planimeter. Each area was traced ten times and the mean recorded for each fiber. This procedure insured better accuracy than relying on one measurement alone. Immediately after each fiber was measured, it was numbered. This procedure reduced the possibility of repeating or omitting fibers. A total myelinated fiber count for each nerve was recorded from the above procedure.

Statistical Procedures

The training data were analyzed by treatment groups and training days. Means, standard deviations and simple correlation coefficients were calculated for training performance, environmental conditions and pre- and post-treatment body weights.

Fiber calibre data were tabled and plotted by treatment and duration. Analysis of contingency tables (ACT) were used to determine if there were any significant differences in distribution between selected pooled groups of animals. Pooling of data was made necessary by the small number of experimental units in many of the cells. (See Chapter IV for details.)

An alpha level of .05 was required to denote statistical significance for all comparisons.

Treatment Results

On the basis of the programmed values of TER for the CRW treatments (Appendix A), animals of the LON group should have had a steady increase in TRR over the training period. The SHT group, however, was expected to have displayed a slight increase in TRR followed by a gradual decrease. It can be seen from Figure 1 that the two running groups met their respective program requirements.

The SHT and LON animals generally exceeded the PER criteria of 75 percent set as a minimum standard for execution of the CRW programs (Figures 2 and 3). This level of performance compares favorably with other groups of animals subjected to similar training programs (36, 61, 78). The animals generally responded to the light stimulus rather than to the electrical shock as reflected in the observed high PSF values (Figures 1 and 2). Comparisons across treatment durations of PSF values for SHT and LON groups showed that the SHT animals received more electrical shock than the LON animals.

The PET values for the SWM group were invariably 100% (Table B-2, Appendix B). Consequently, PET values were not plotted across duration for the SWM animals.

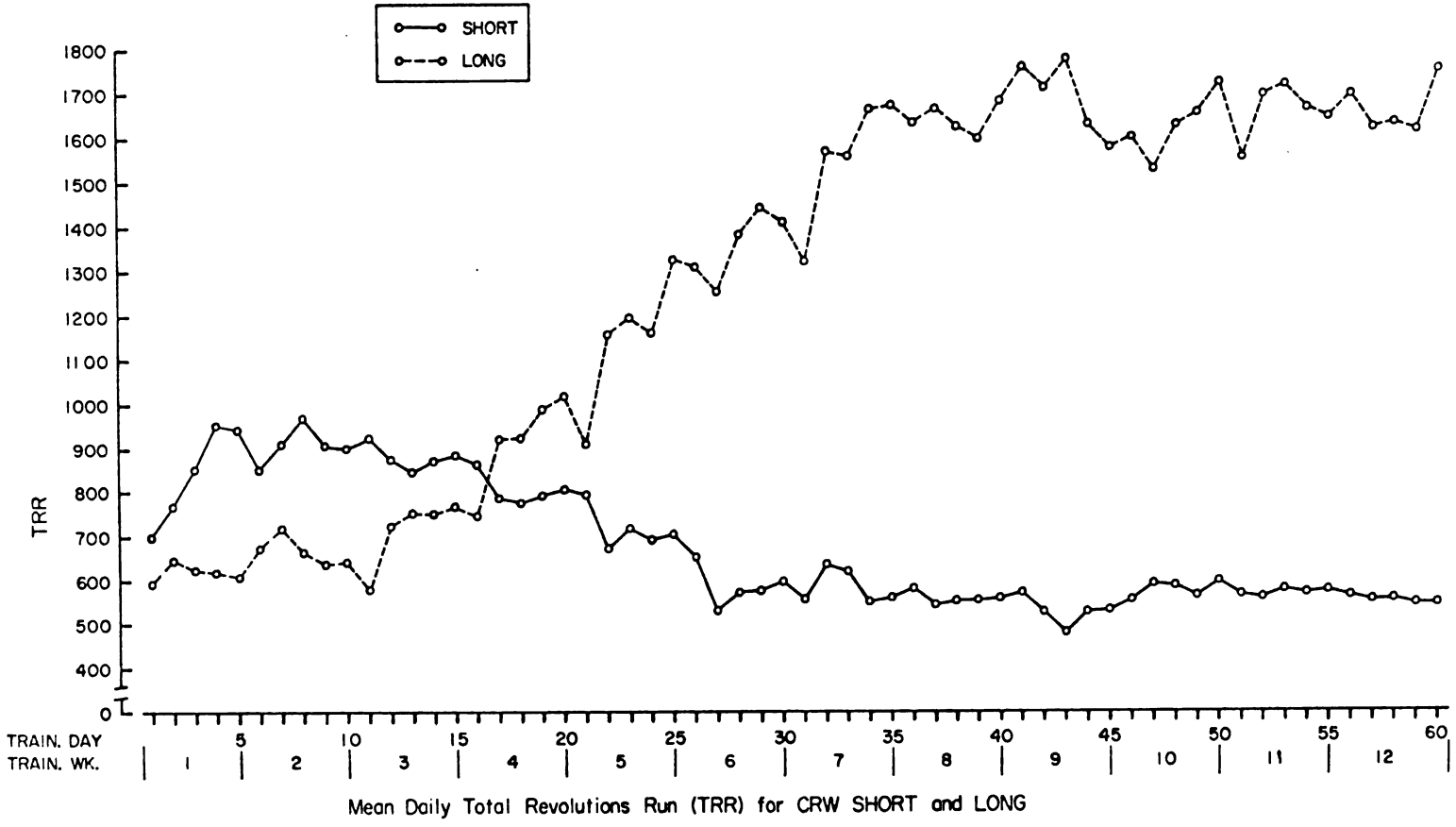
Treatment Environment and Body Weight Results

The CRW animals were exercised under relatively constant conditions of air temperature, humidity and barometric pressure. These variables did not affect the PER and PSF values as reflected by the low correlations among the parameters (Table B-1, Appendix B). A moderate inverse relationship existed between pre-treatment body weight and PER. That is, animals with high pre-treatment body weights tended to display low PER values. The moderate positive correlation between PSF and PER confirms the nearly parallel plots of these two values (Figures 1 and 2).

The SWM animals were exercised under controlled conditions comparable to the CRW animals (Table B-2, Appendix B). In addition, the water temperature was regulated at approximately 32° C. None of the environmental or pretreatment body weight values were highly correlated with PET.

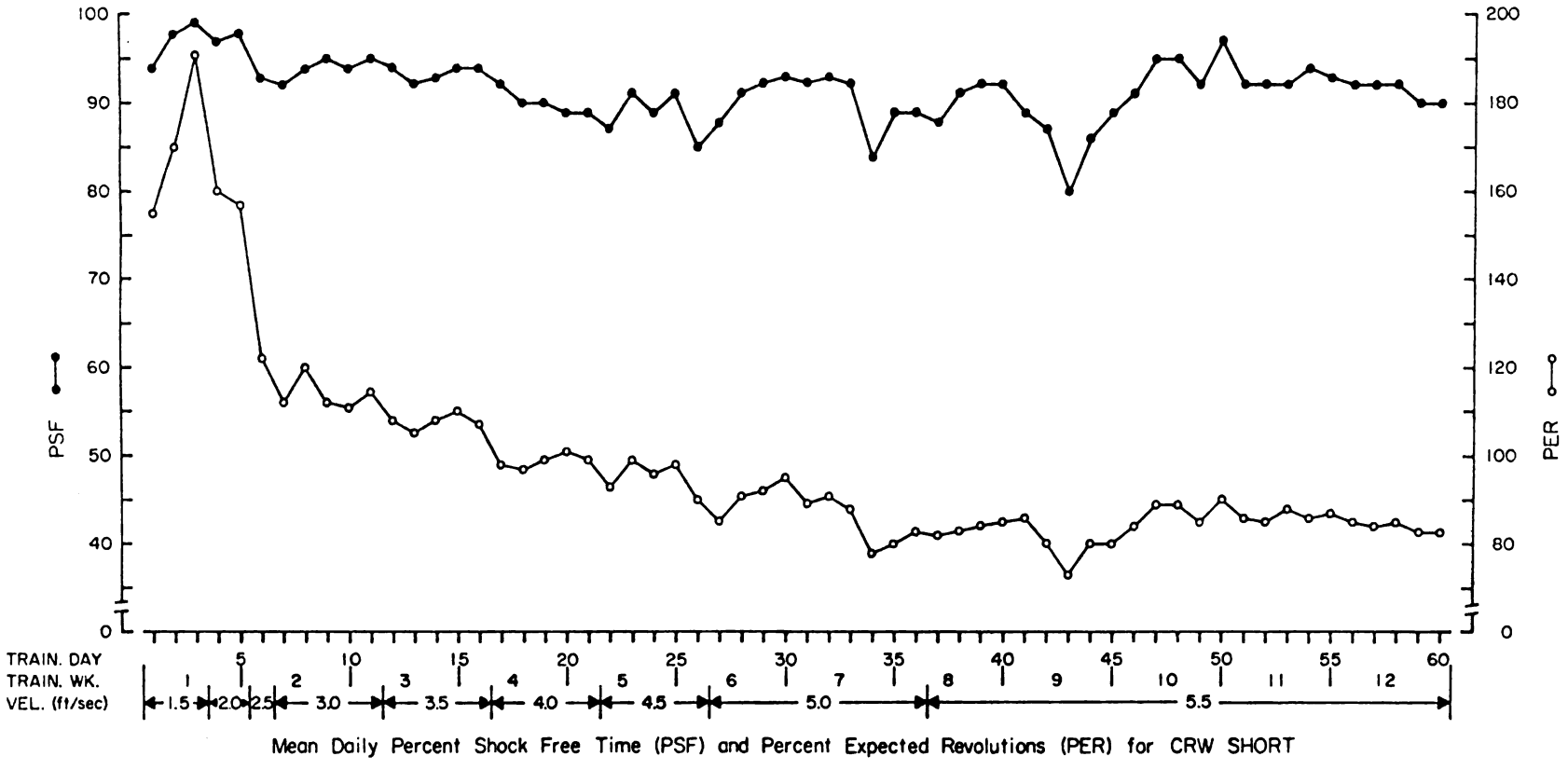
Training Results

Five distinct types and levels of chronic physical activity were utilized in the study. The CON animals were sedentary throughout the experimental period and represented the lowest level of activity. Similarly, the ESC animals were not subjected to any scheduled training regimen. However, the response of these animals to the noxious stimuli distinguished the ESC animals from those in the CON group. The SHT and LON running programs resulted in mean daily TRR values which were markedly different (Figure 1). Program expectations for the CRW and SWM groups differentiated the trained groups into three separate categories (Appendix A).



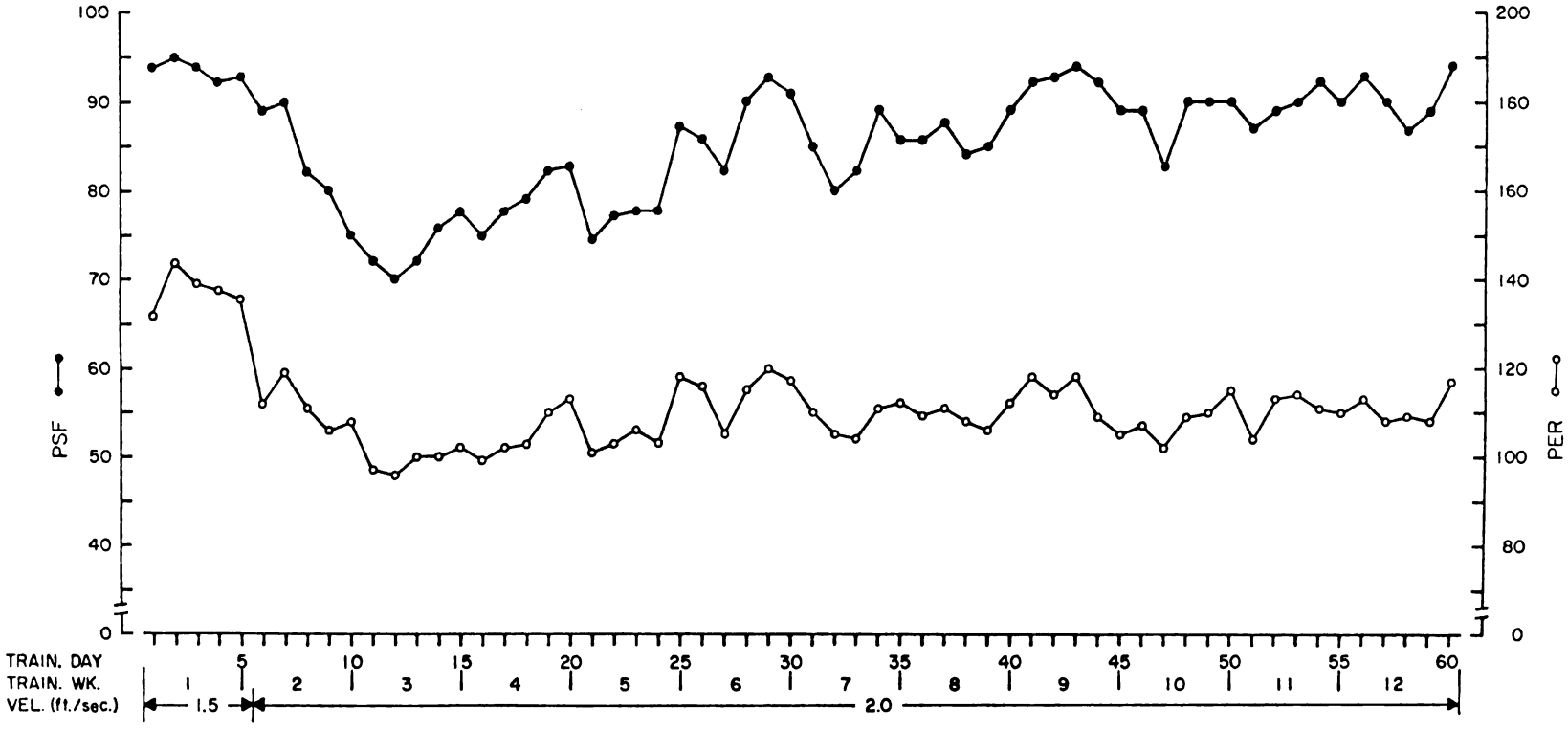
Mean Daily Total Revolutions Run (TRR) for CRW SHORT and LONG

Figure 1



Mean Daily Percent Shock Free Time (PSF) and Percent Expected Revolutions (PER) for CRW SHORT

Figure 2



Mean Daily Percent Shock Free Time (PSF) and Percent Expected Revolutions (PER) for CRW LONG

Figure 3

CHAPTER IV

RESULTS AND DISCUSSION

The morphological data will be presented and analyzed separately for each independent variable: myelin area, axon area and total area. Statistical procedures utilizing a system of pooling techniques will be summarized in the second part of the chapter. A brief section on the findings involving fiber counts will follow. Finally, a discussion attempting to relate the morphological findings with physiological and related processes will be offered.

Morphological Results

The distributions of the three dependent variables were plotted and are presented by percent frequency: (a) for each experimental treatment across duration (Figures 4 to 18), and (b) for each duration across treatment (Figures 19 to 27). This procedure was followed in an attempt to better illustrate the general trends and shifts in fiber distributions by treatments and durations.

Due to limitations in measurement and recording techniques, extreme fiber sizes were grouped at the upper and/or lower ends of the distributions. Bar graphs are used to represent the grouped values. Percent frequencies above eight percent are indicated by broken bars. In no instance does the grouping technique involve more than seven, nine or twenty-four percent of the total number of fibers in the myelin, total or axonal plots respectively.

Pooling of all zero-week animals was utilized as presented in Chapter III. Consequently zero-week graphs are identical across treatments for each dependent variable. For example, zero-week graphs are the same for total area in Figures 14 to 18.

Myelin Area

The control group showed a general increase in myelination across duration (Figure 4). The bimodal distribution evident at zero-weeks duration flattened out with time. Shifts in the distribution to the right were evident at both modes. This observation might have been anticipated as a direct result of maturation and normal growth processes.

The SHT CRW program resulted in an initial shift to the right at four weeks followed by dramatic shifts to the left at eight- and twelve-weeks duration (Figure 5). The multipeaked effect seen at twelve weeks is inexplicable but showed up in many of the final duration graphs. The ESC plots were similar to those of the SHT group (Figure 7).

At the end of twelve weeks of training, the LON group demonstrated shifts towards both ends of the curve (Figure 6). A larger percentage of both small and large fibers were present at twelve weeks than at zero weeks.

Shifts to the left were evident in the SWM data at four and eight weeks (Figure 8). By twelve weeks, the curve was similar to that at zero weeks.

Comparisons across treatment by duration revealed additional trends in the distributions (Figures 17 to 21). At four weeks, the SHT and ESC groups showed shifts towards larger myelin areas while the LON and SWM distributions shifted to the left. After twelve weeks of training, all treatment groups had an increased percentage of small

myelinated fibers. This outcome was most evident in the SHT and ESC groups. The LON distribution was the only one which showed any increase at the upper end of the curve.

The tendency was for the power-type anaerobic treatment group (SHT) to have the largest increase in percentage of small fibers across duration. The ESC group showed similar patterns. Conversely, the endurance-type aerobic activity programs resulted in distributions resembling the control group. Shifts to larger myelin areas were more evident within the LON group than in the SWM group. However, both had fewer small fibers than either the SHT or ESC groups.

Axon Area

Axon areas of the control group tended to shift slightly to the left at four-weeks duration and then shift back to the right at eight and twelve weeks (Figure 9). At twelve weeks, the shift to the right was evident at both the upper and the lower ends of the curve. The small number of fibers at the extreme upper end of the curve possibly could be accounted for by artifact.

The SHT group plots demonstrated a shift to the right at four weeks, an extreme reversal at eight weeks, and a less dramatic shift back to the right at twelve weeks (Figure 10). The ESC graphs followed a similar pattern except that the eight-week shift is much less prominent (Figure 12). (Note the multipeaked curves at twelve weeks duration.)

The two long-endurance groups had similar patterns (Figures 11 and 13). A large percentage of small fibers are evident at four and eight weeks. Prominent shifts back to the right and curves resembling zero-

week data are observed at the end of the training programs. Extremely small changes are seen at the upper end of the curves for either the LON or the SWM groups.

At four-weeks duration, the SHT and ESC graphs had definite shifts to the right at both ends of the curve (Figure 22). The LON and SWM groups showed an increase in small axon areas and a very slight increase at the large end of the size spectrum. By eight weeks, all treatment groups had a much larger percentage of small axons than did the control group (Figure 23). The ESC group also showed a slight increase in large axonal areas. The twelve-week plots are similar to the eight-week curves (Figure 24). The highly peaked nature of the SHT, ESC and SWM distributions are notable.

The control group demonstrated a shift towards larger axonal areas with time (Figures 22 to 24). Therefore a time related increase in axonal size was assumed to be operating.

All experimental treatments resulted in an increased percentage of small fibers. This was most evident for the SHT and ESC groups.

Total Area

The total area is presented as an indicator of the effects of exercise on the combined areas of the axon and the myelin sheath. Several previous investigations have limited their morphological analyses to this single index (5, 25, 82, 85). Interpretation of the graphs is more difficult than for the previous measures due to the broadness and flatness of the curves.

The control treatment graphs indicated a shift to the right across duration (Figure 14). This effect was found at both ends of the curve. All experimental groups showed definite shifts to the left (Figures 15 to 18).

For the SHT, LON and SWM groups, the shift towards small fibers was most evident at eight weeks (Figure 26). A slight shift back to the right was seen at both ends of the graphs after twelve weeks of training (Figure 27). A peculiarity in the ESC graphs is seen at four and eight weeks. A large percentage of fibers are grouped at the extreme upper end of the scale. No explanation can be offered for this observation.

Pooling Techniques and Statistical Results

Difficulties in the preparation techniques described in Chapter III resulted in gross reductions of the sample size. Consequently, the use of pooling techniques was seen as a necessity in presenting and interpreting the morphological data.

In an attempt to determine if the exercise regimens did alter the population of neurons in the nerve, the data were pooled and analyzed in two ways. First, the distribution of fibers of the control animals at each duration was compared with the distribution of fibers of the four other treatments pooled within duration. For example, the four-week CON data were compared with the four-week SHT, ESC, LON and SWM data pooled.

Table 2 indicates that the fiber distributions of the trained animals (TR) were significantly different from the controls for all three variables at the end of twelve weeks. A change in axon area found at four weeks disappeared at eight weeks and then reappeared at twelve weeks. No attempt was made to explain this phenomenon.

A second way to extract the exercise effect was to pool treatments across durations and compare these results to those of the controls

pooled across duration. For example, the four-, eight- and twelve-week CON data were pooled and compared with the four-, eight- and twelve-week SHT pooled data.

Table 2. Summary of analyses of chi square contingency tables between controls and pooled experimental groups within duration for axon, myelin and total areas

Comparisons	Axon	Myelin	Total
4-wk CON vs 4-wk TR	S	N	N
8-wk CON vs 8-wk TR	N	S	S
12-wk CON vs 12-wk TR	S	S	S

N = not significant.
S = significant distribution difference at the .05 level.
TR = a pooled distribution of SHT, LON, ESC, and SWM data.

The results summarized in Table 3 indicate that the pooled fiber caliber distributions of the individual treatment groups are significantly different from the pooled distribution of the controls in all cases except two. No attempt has been made to explain these exceptions.

Table 3. Summary of analyses of chi square contingency tables between controls and individual treatments pooled across durations for axon, myelin and total areas

Comparisons	Axon	Myelin	Total
4-8-12 wk CON vs 4-8-12 wk SHT	S	S	S
4-8-12 wk CON vs 4-8-12 wk LON	N	S	S
4-8-12 wk CON vs 4-8-12 wk ESC	S	S	N
4-8-12 wk CON vs 4-8-12 wk SWM	S	S	S

N = not significant.
S = significant distribution difference at the .05 level.

The possibility of a duration effect has been pursued in two manners. First, the comparisons between the zero-week pooled distribution and the individual treatments pooled across duration were analyzed. For example, the zero-week pooled distribution was compared with the four-, eight- and twelve-week CON pooled distribution.

The results of this analysis showed a significant change in myelin distributions across durations for all activity levels including the CON group (Table 4). All total area comparisons except for the CON group also were significant. However, axonal changes were evident only within the SHT and ESC groups. The implication of a possible specific treatment-duration interaction effect, inherent in this data, was the major impetus for the following second method of analysis.

Table 4. Summary of analyses of chi square contingency tables between the zero-week pooled distribution and the distributions for individual treatments pooled across duration for axon, myelin and total areas

Comparisons	Axon	Myelin	Total
0 wk P vs 4-8-12 wk CON	N	S	N
0 wk P vs 4-8-12 wk SHT	S	S	S
0 wk P vs 4-8-12 wk LON	N	S	S
0 wk P vs 4-8-12 wk ESC	S	S	S
0 wk P vs 4-8-12 wk SWM	N	S	S

N = not significant.
S = significant distribution difference at the .05 level.
P = pooled.

The zero-week pooled distributions were compared with the distributions for each individual treatment-duration cell (Table 5). Since the frequency

for each individual cell was either one or two, the power of the statistical analyses are limited. However, general patterns may be derived from such comparisons.

Table 5. Summary of analyses of chi square contingency tables between the zero-week pooled distributions and the distributions for each individual treatment-duration cell for axon, myelin and total areas

Comparisons	Axon	Myelin	Total
0-wk P vs 4-wk CON	N	N	N
0-wk P vs 8-wk CON	N	S	S
0-wk P vs 12-wk CON	N	S	S
0-wk P vs 4-wk SHT	S	S	S
0-wk P vs 8-wk SHT	S	S	S
0-wk P vs 12-wk SHT	S	S	S
0-wk P vs 4-wk LON	N	N	S
0-wk P vs 8-wk LON	N	S	S
0-wk P vs 12-wk LON	N	S	S
0-wk P vs 4-wk ESC	S	S	S
0-wk P vs 8-wk ESC	S	S	S
0-wk P vs 12-wk ESC	S	S	N
0-wk P vs 4-wk SWM	N	S	S
0-wk P vs 8-wk SWM	N	S	S
0-wk P vs 12-wk SWM	N	S	N

N = not significant.
S = significant distribution difference at the .05 level.
P = pooled.

As could be expected from the previous analyses, the myelin area distributions of the separate treatment groups were generally significantly different from the zero-week pooled distribution at all durations. At the end of eight weeks, all comparisons were significantly different. In contrast, the axonal area distributions exhibited specificity. That is, the CON, LON and ESC groups showed no changes from the zero-week pooled distribution across durations. However, the SHT and ESC groups demonstrated significant alterations in axonal size at each duration. The total area comparisons masked this specificity

phenomenon and resulted in significant differences in most cases.

Total Fiber Numbers

Total myelinated fiber counts were recorded for each animal. Table 6 summarizes the observed results.

Table 6. Individual cell means for total fiber numbers

Treatment	Duration			
	0-wk.	4-wk.	8-wk.	12-wk.
CON	102[1](5)[2]	115(2)	120(2)	107(2)
SHT		107(2)	116(2)	106(2)
LON		103(2)	107(2)	109(2)
ESC		103(2)	118(2)	92(1)
SWM		125(1)	94(1)	100(2)

[1]Mean fiber count in each cell.

[2]Number of animals in each cell.

Since the sample size was extremely limited, no statistical analyses were performed. However, some general patterns are evident in the data. In most instances, the treatment groups had fewer myelinated fibers than the control group at any specific duration. Across durations, the CON, SHT and ESC groups demonstrated similar trends: a slight increase at four weeks followed by a larger increase at eight weeks and a dramatic decrease at twelve weeks. No explanations are attempted for these phenomena.

Discussion

Several trends in the morphological data have been presented in the results sections. In the following discussion, an attempt has

been made to organize these trends into correlated patterns and to offer possible explanations for the findings.

The control group demonstrated increases in myelin, axon and total areas across duration. Normal maturation and growth processes have been considered as the probable causes of these results. Total fiber counts followed a similar pattern.

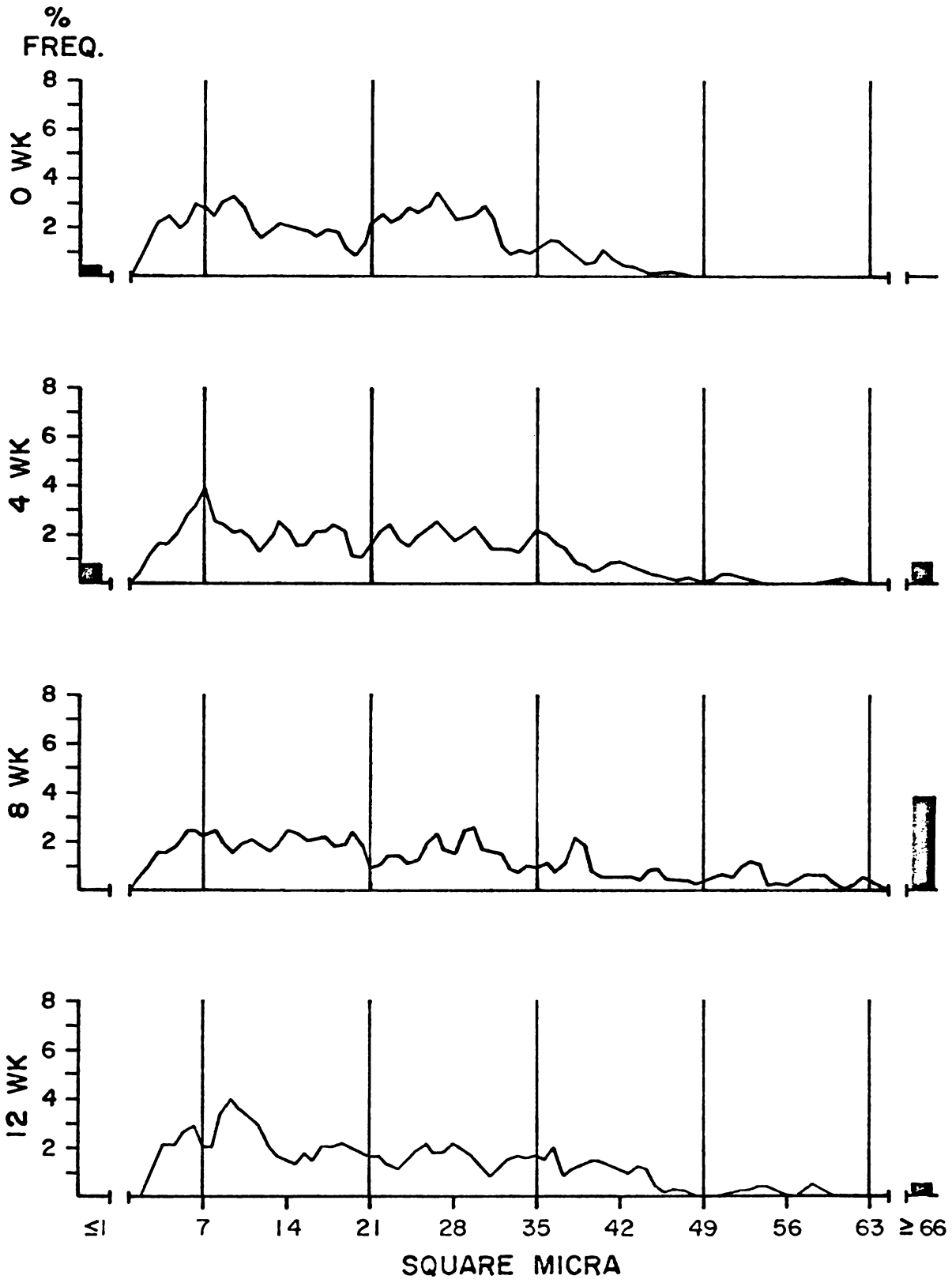
Most experimental group graphs indicate shifts towards smaller areas for the dependent variables across durations. The SHT and ESC groups showed the most notable decreases. A possible specific effect of power-type anaerobic exercise is postulated as the cause of these extreme reductions in size. The likelihood of such an effect is enhanced by a closer examination of the data for the electrical stimulus control group. The ESC animals may, in fact, have performed a power-type isometric exercise rather than acting simply as electrical controls. Thus, the similarities in recorded data for the SHT and ESC groups may be due to the participation of both groups at an anaerobic activity level.

Chi square analyses reflected significant alterations in fiber distributions across treatments and durations. The system of pooling utilized did not negate the fact that observable differences existed within treatments and durations which were made obvious by the plots.

Speculations as to the causes of these alterations in the size of myelinated fibers are innumerable. However, mechanisms involved in the normal process of nerve growth must certainly be investigated. As defined by Weiss (86), enlargement, or true growth, of the nerve fiber is the result of the production of new protoplasm at a rate which exceeds the elongation or "towing" process and increases the width of the axon.

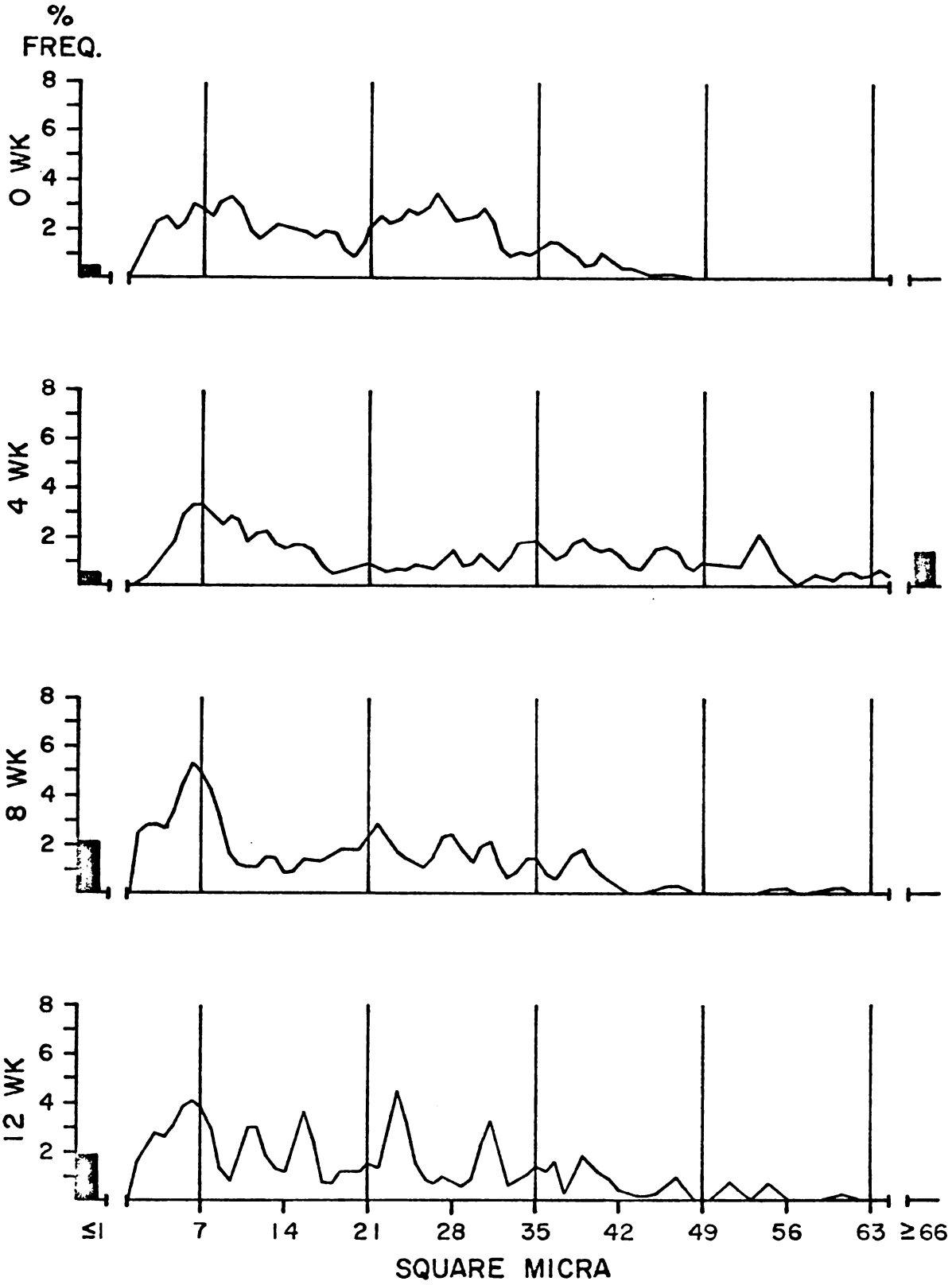
In their classical series of nerve regeneration and constriction studies (93), Weiss and Hiscoe ultimately concluded that the caliber of a nerve fiber essentially is determined by two factors: the amount of synthesis of new axoplasm in the cell body and the rate of its centrifugal movement (ie: axoplasmic transport). The effects of specific levels of activity on these parameters have yet to be investigated.

A third factor to be scrutinized is the importance of peripheral factors upon the process of nerve growth. Several regenerative studies have demonstrated that the most powerful peripheral influence is the contact which is made with the end organs (3, 4, 24, 28, 67, 68, 95, 96). In the present study, a nerve-muscle interdependence is implied. How specific exercise regimens effect this interrelationship is unproven at this time.



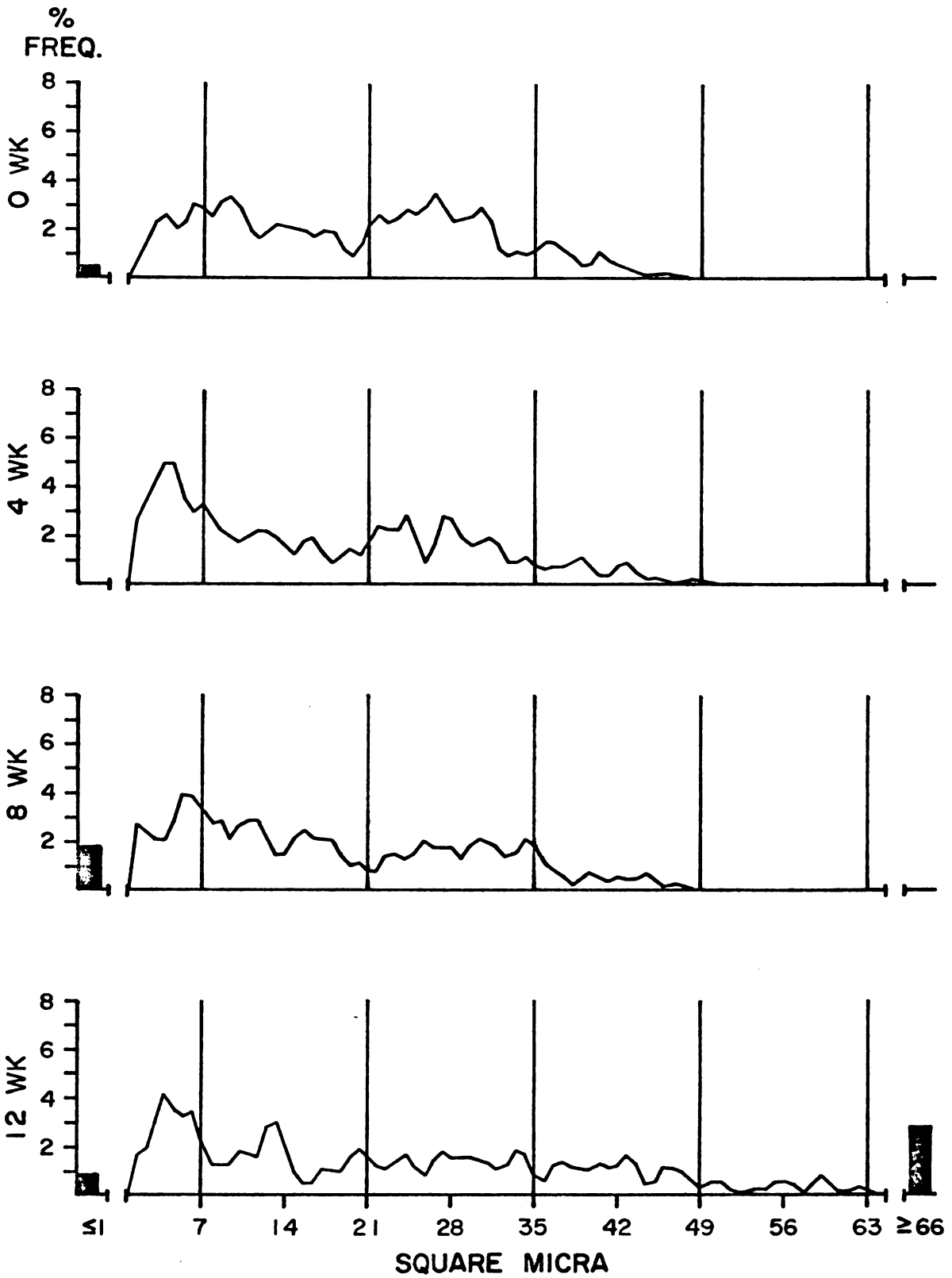
Myelin Area by Percent Frequency for the Control Experimental Treatment across Duration

Figure 4



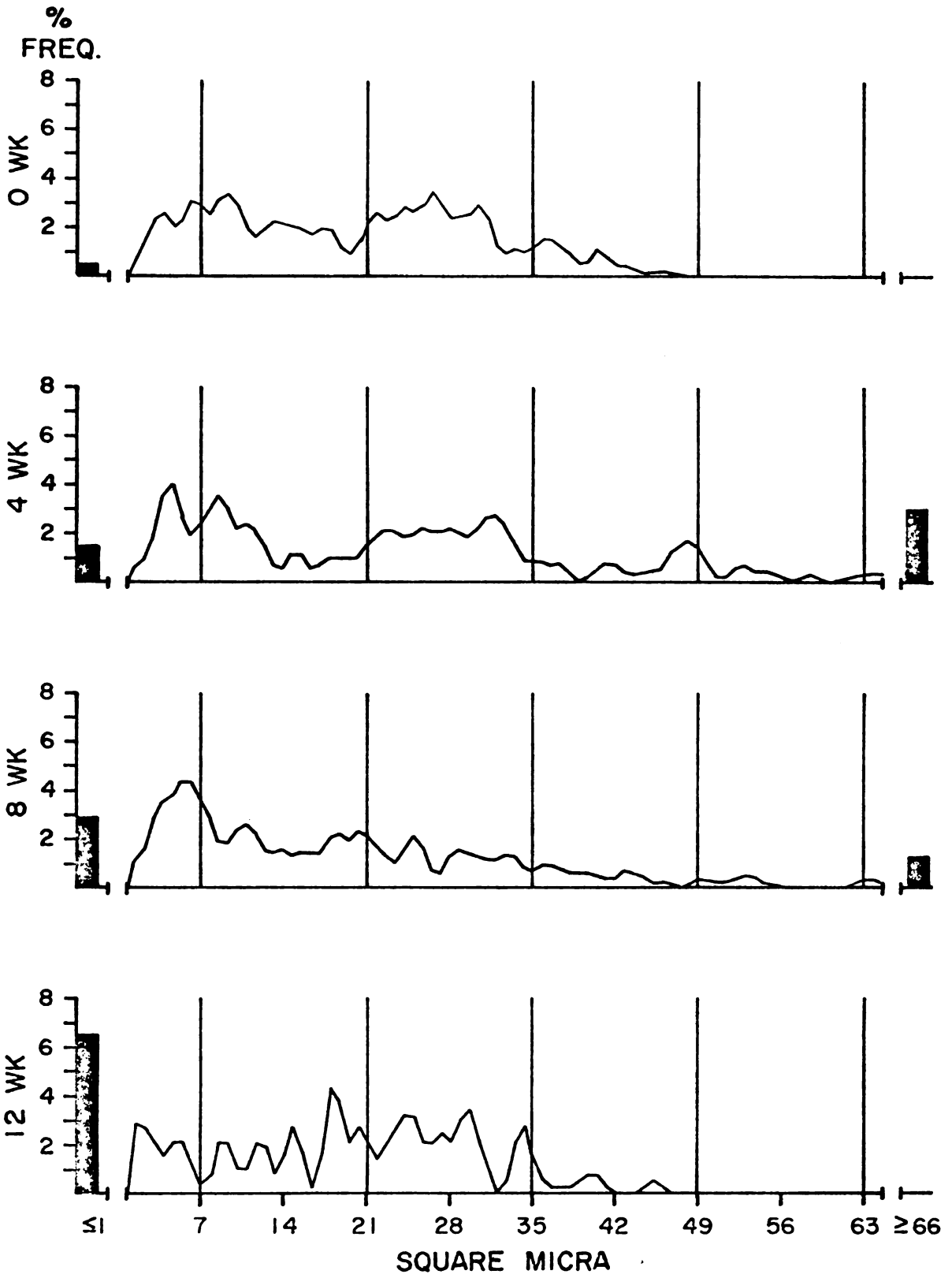
Myelin Area by Percent Frequency for the Short Experimental Treatment across Duration

Figure 5



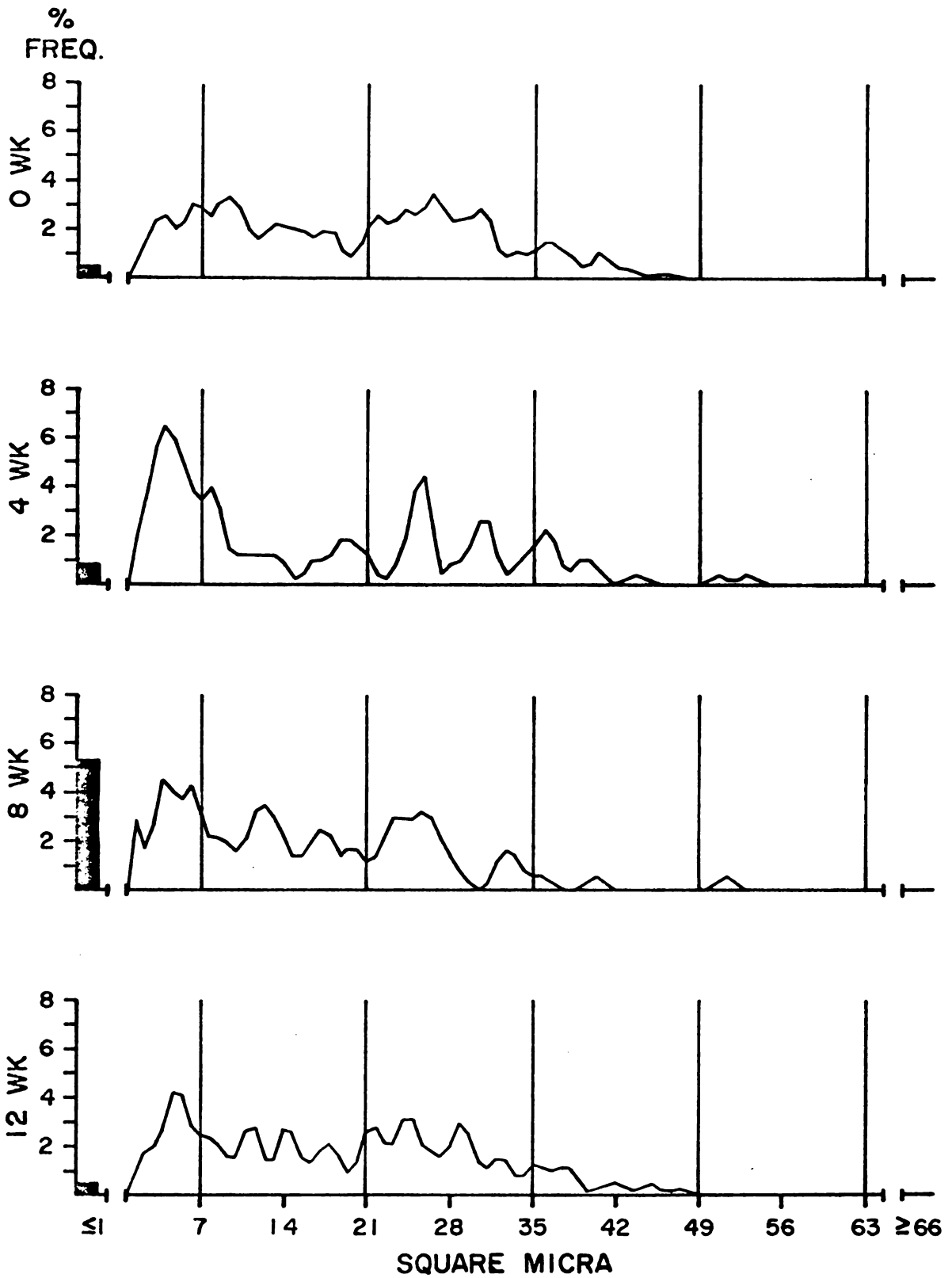
Myelin Area by Percent Frequency for the Long Experimental Treatment across Duration

Figure 6



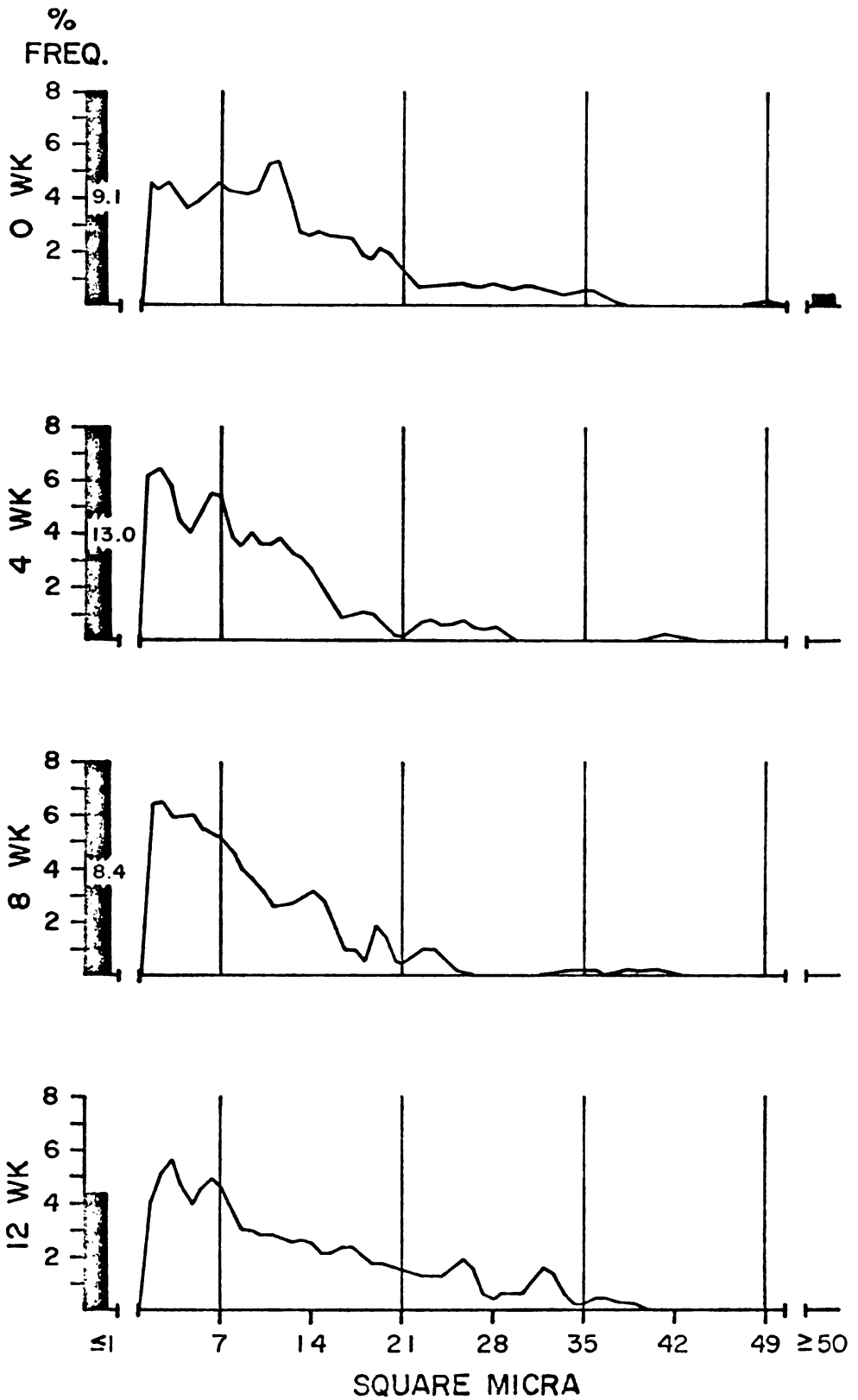
Myelin Area by Percent Frequency for the Electrical Stimulus Control Experimental Treatment across Duration

Figure 7



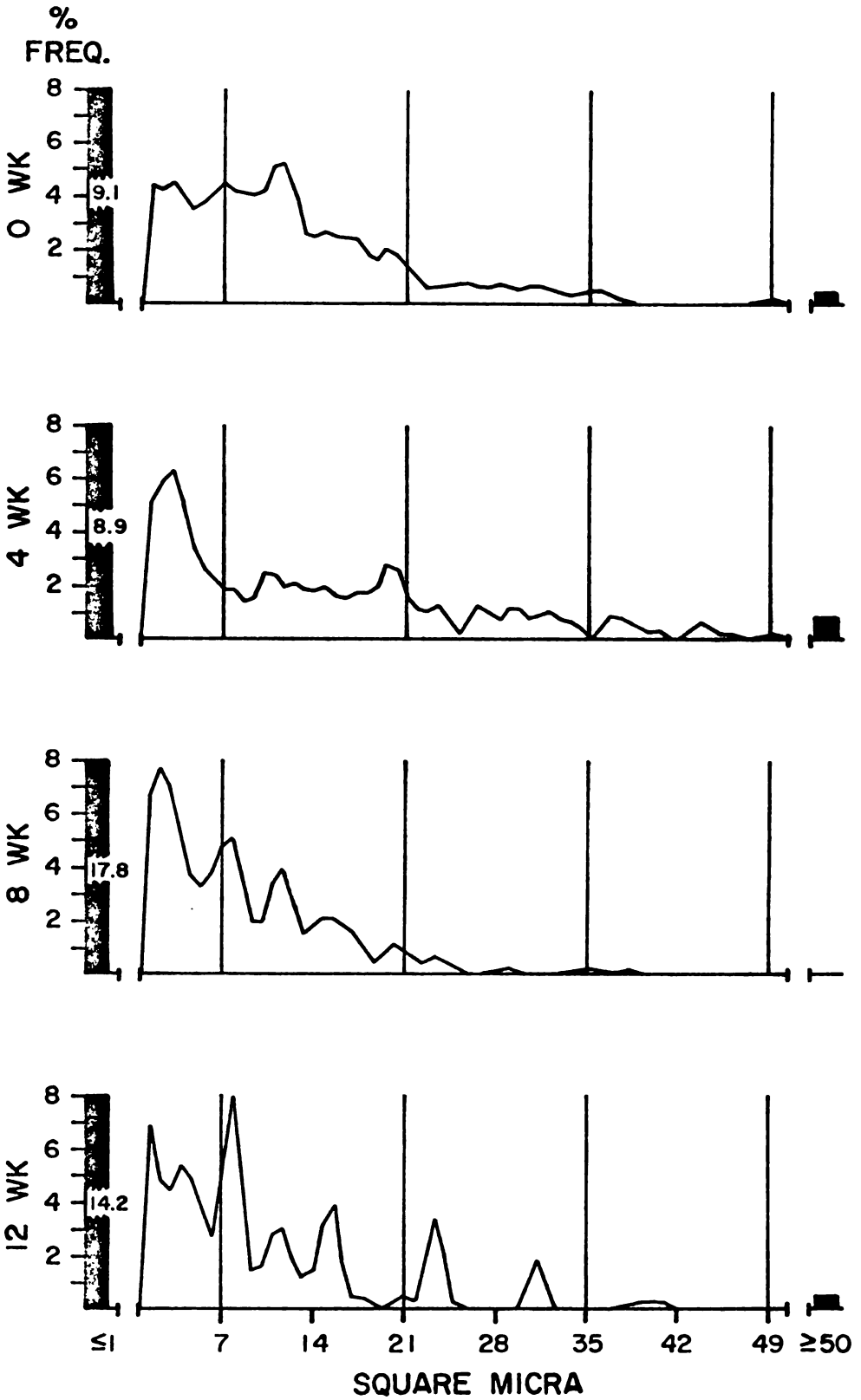
Myelin Area by Percent Frequency for the Swim Experimental Treatment across Duration

Figure 8



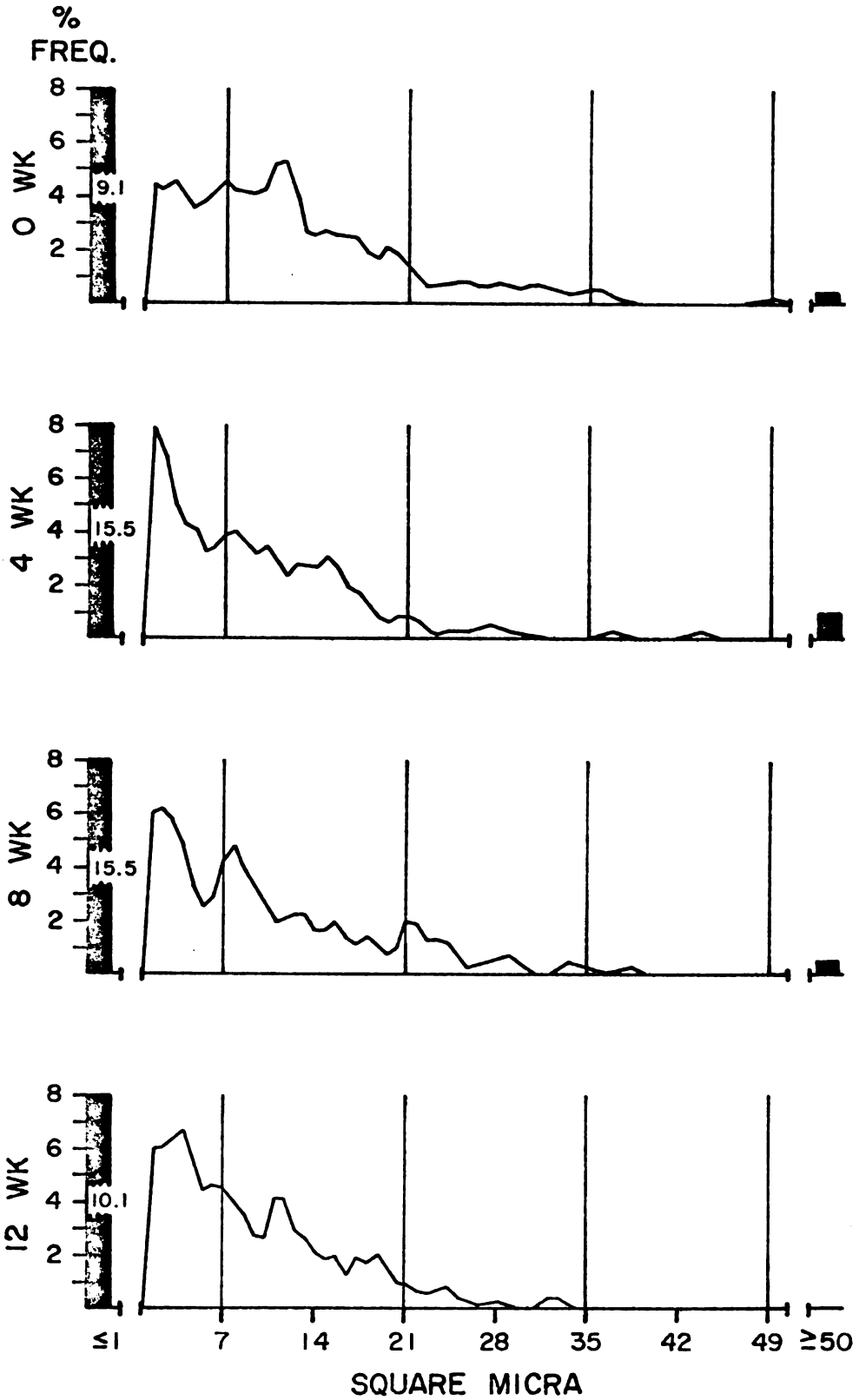
Axon Area by Percent Frequency for the Control Experimental Treatment across Duration

Figure 9



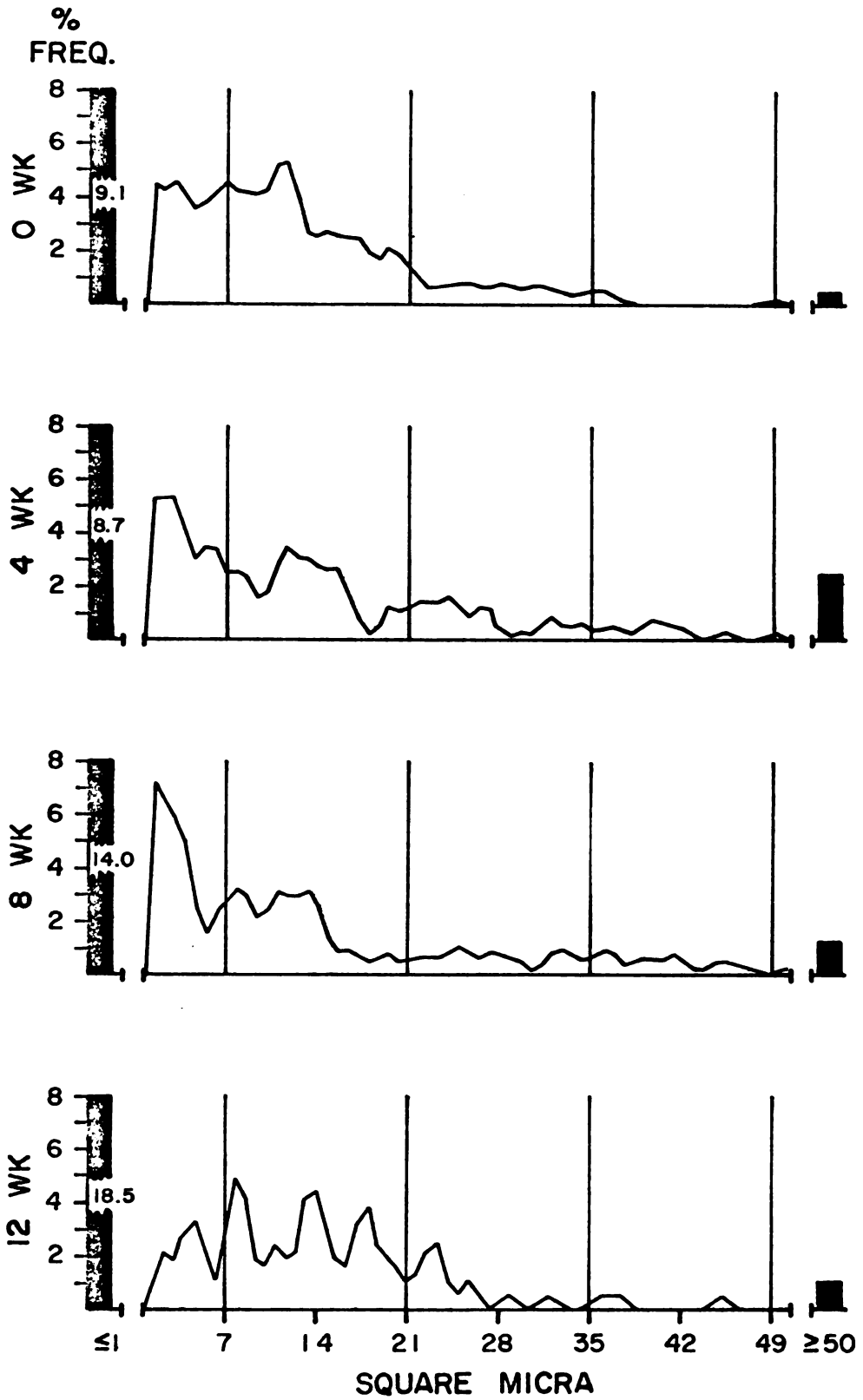
Axon Area by Percent Frequency for the Short Experimental Treatment across Duration

Figure 10



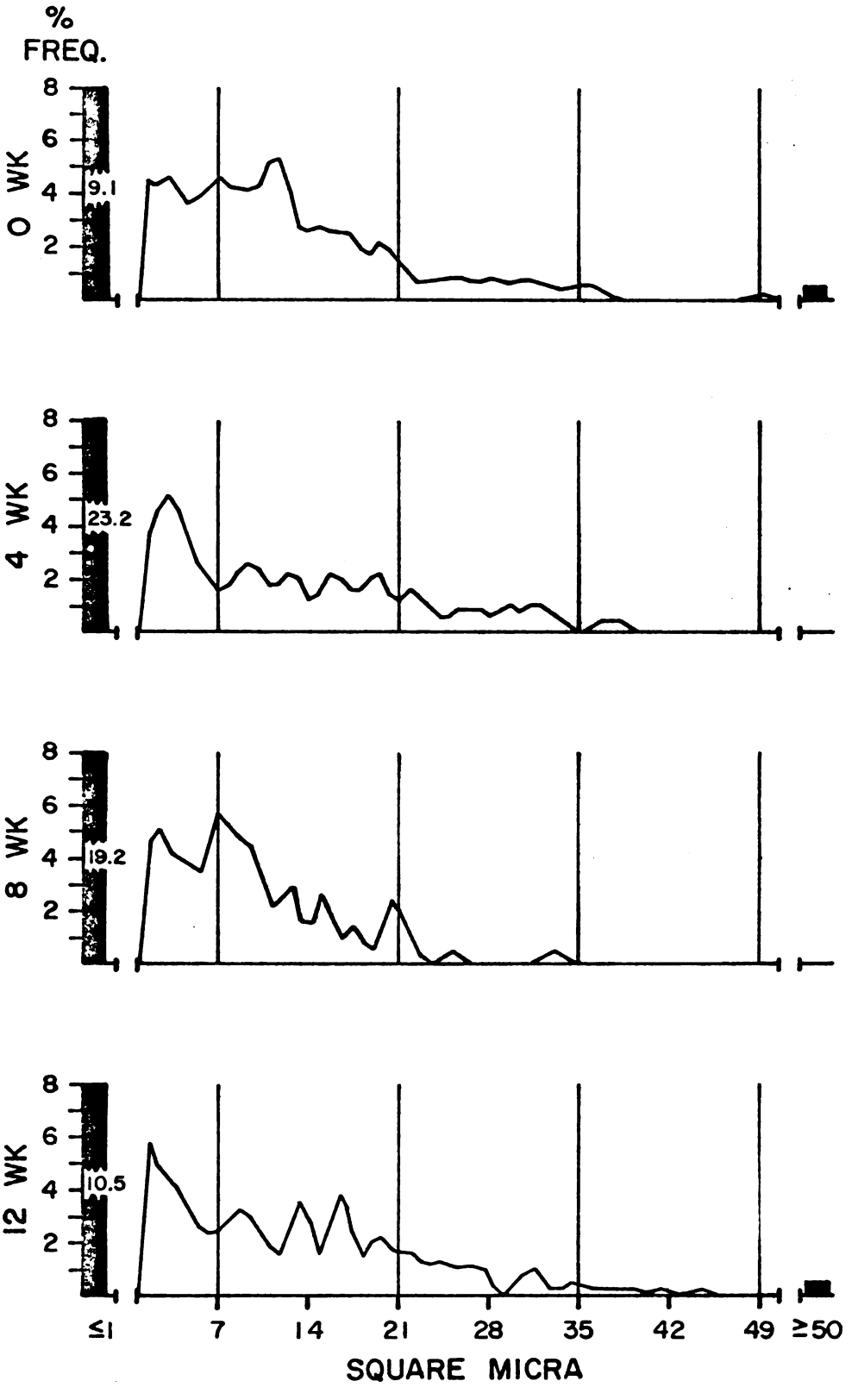
Axon Area by Percent Frequency for the Long Experimental Treatment across Duration

Figure 11



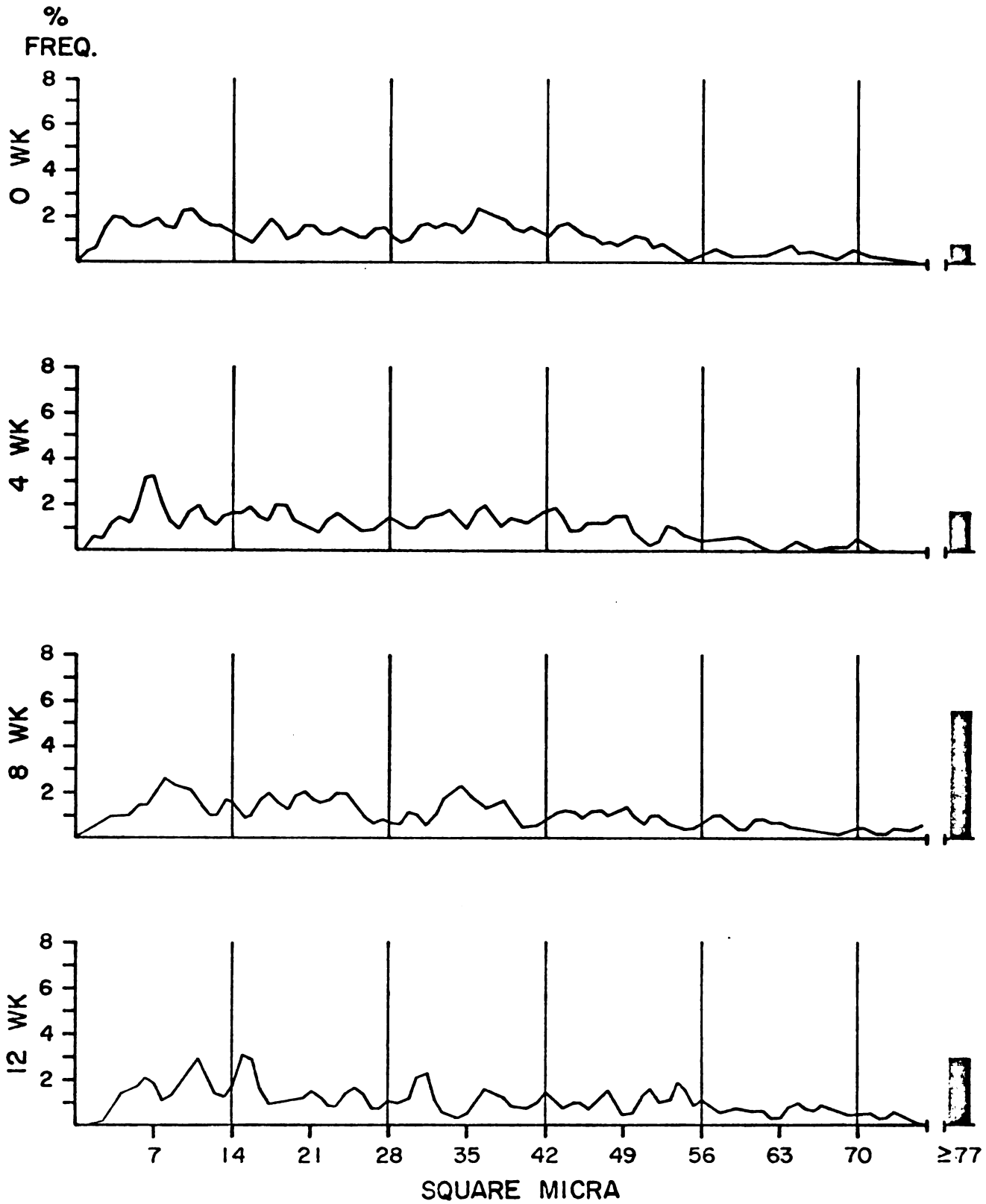
Axon Area by Percent Frequency for the Electrical Stimulus Control Experimental Treatment across Duration

Figure 12



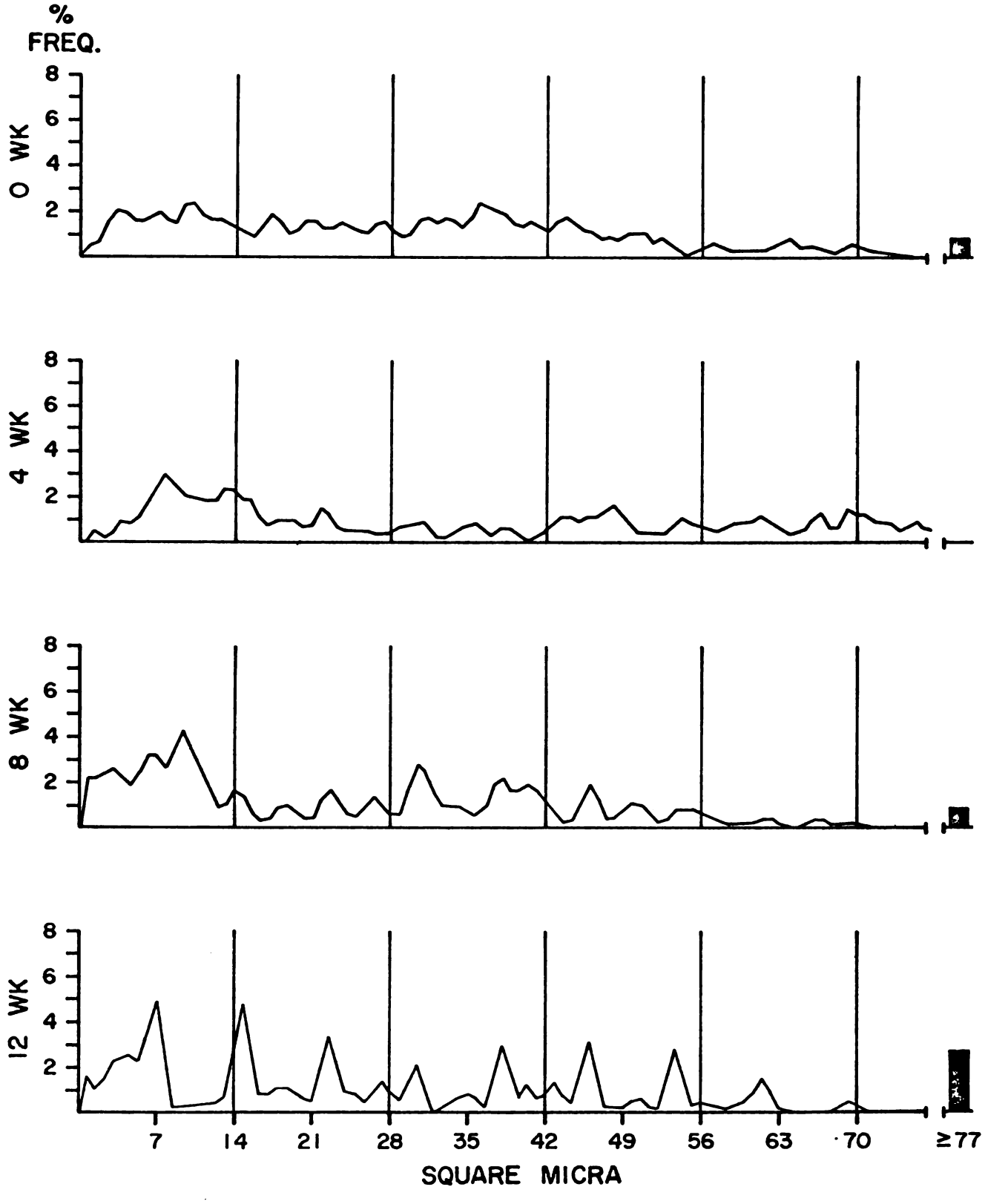
Axon Area by Percent Frequency for the Swim Experimental Treatment across Duration

Figure 13



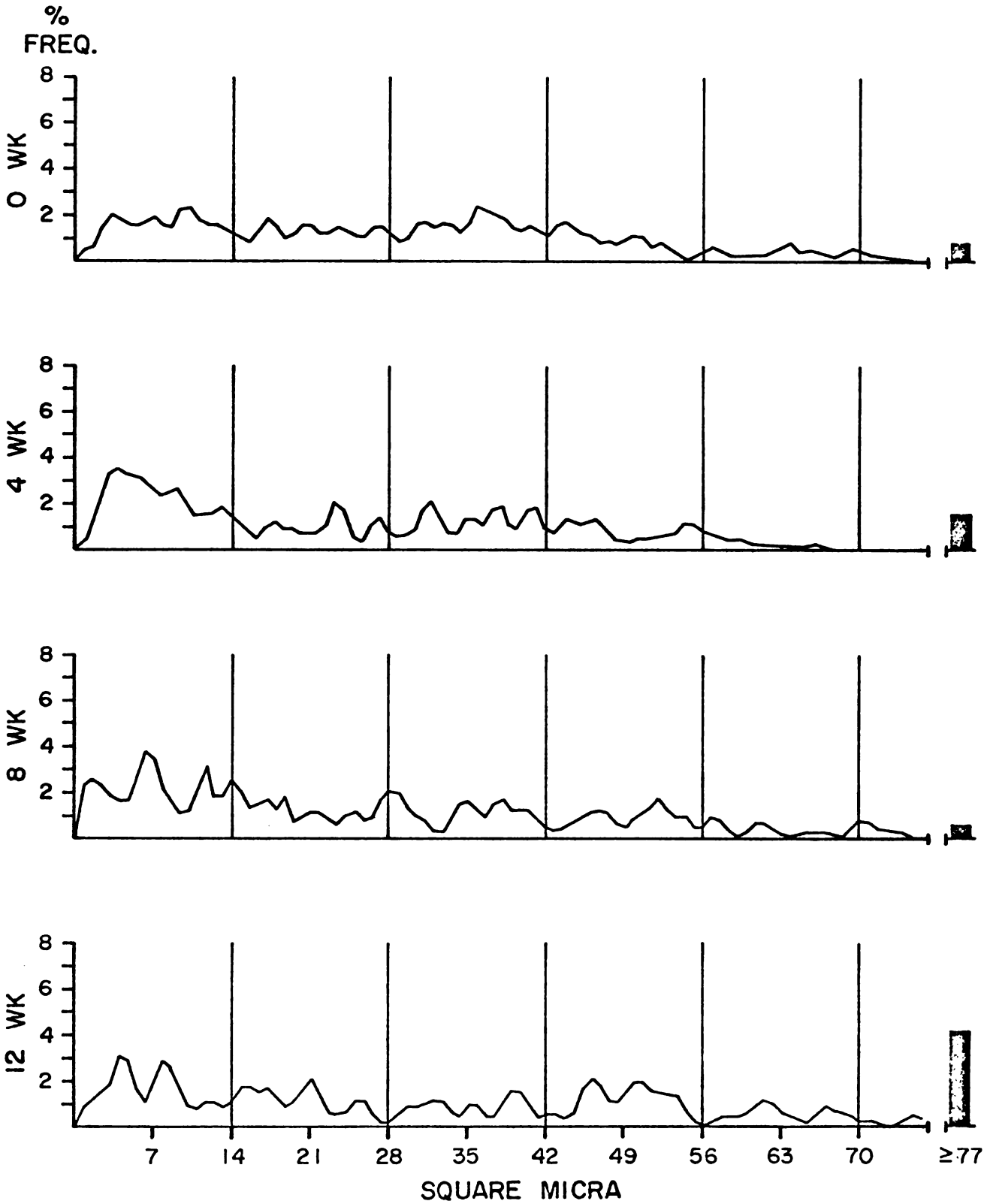
Total Area by Percent Frequency for the
Control Experimental Treatment across Duration

Figure 14



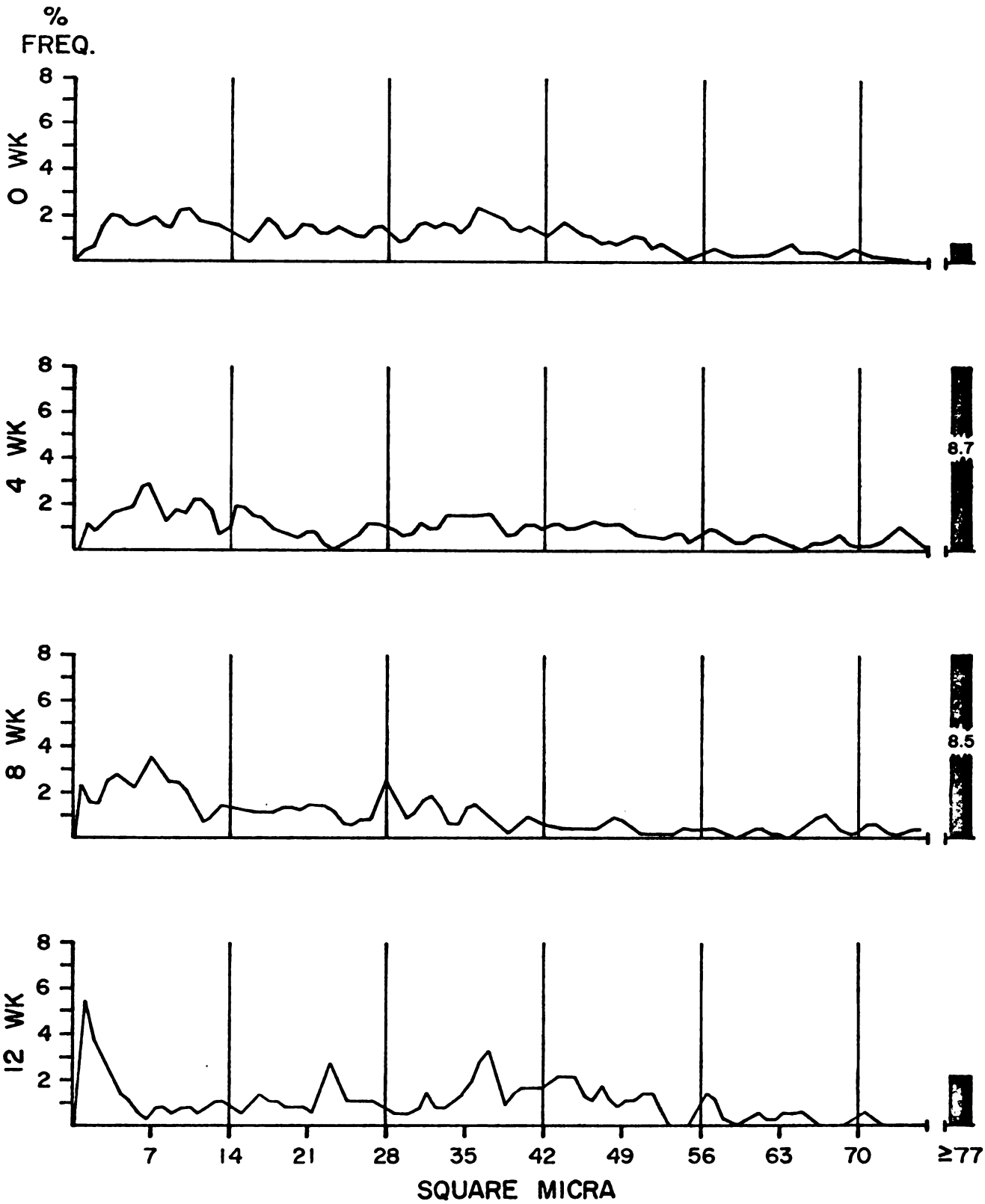
Total Area by Percent Frequency for the
Short Experimental Treatment across Duration

Figure 15



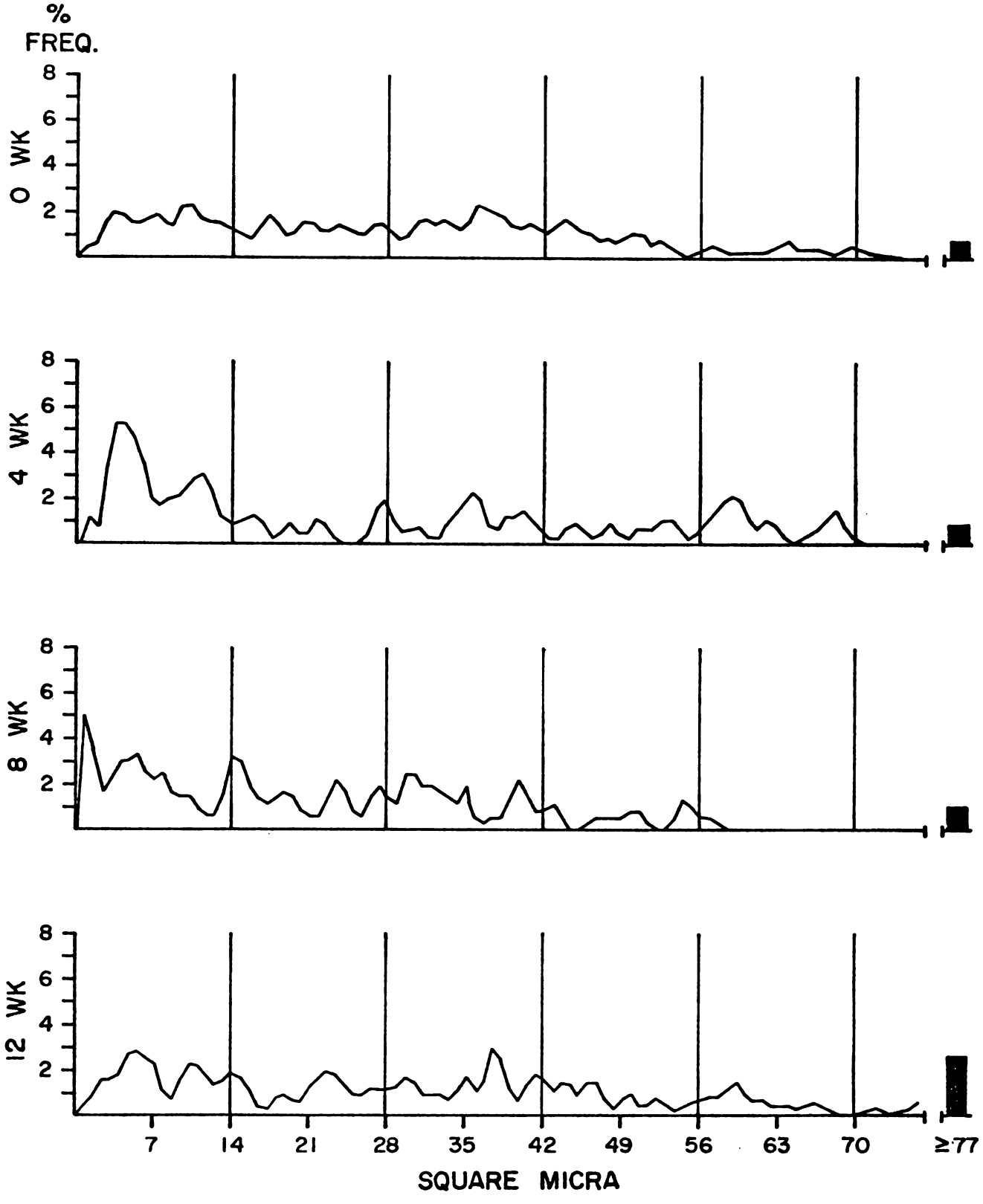
Total Area by Percent Frequency for the Long Experimental Treatment across Duration

Figure 16



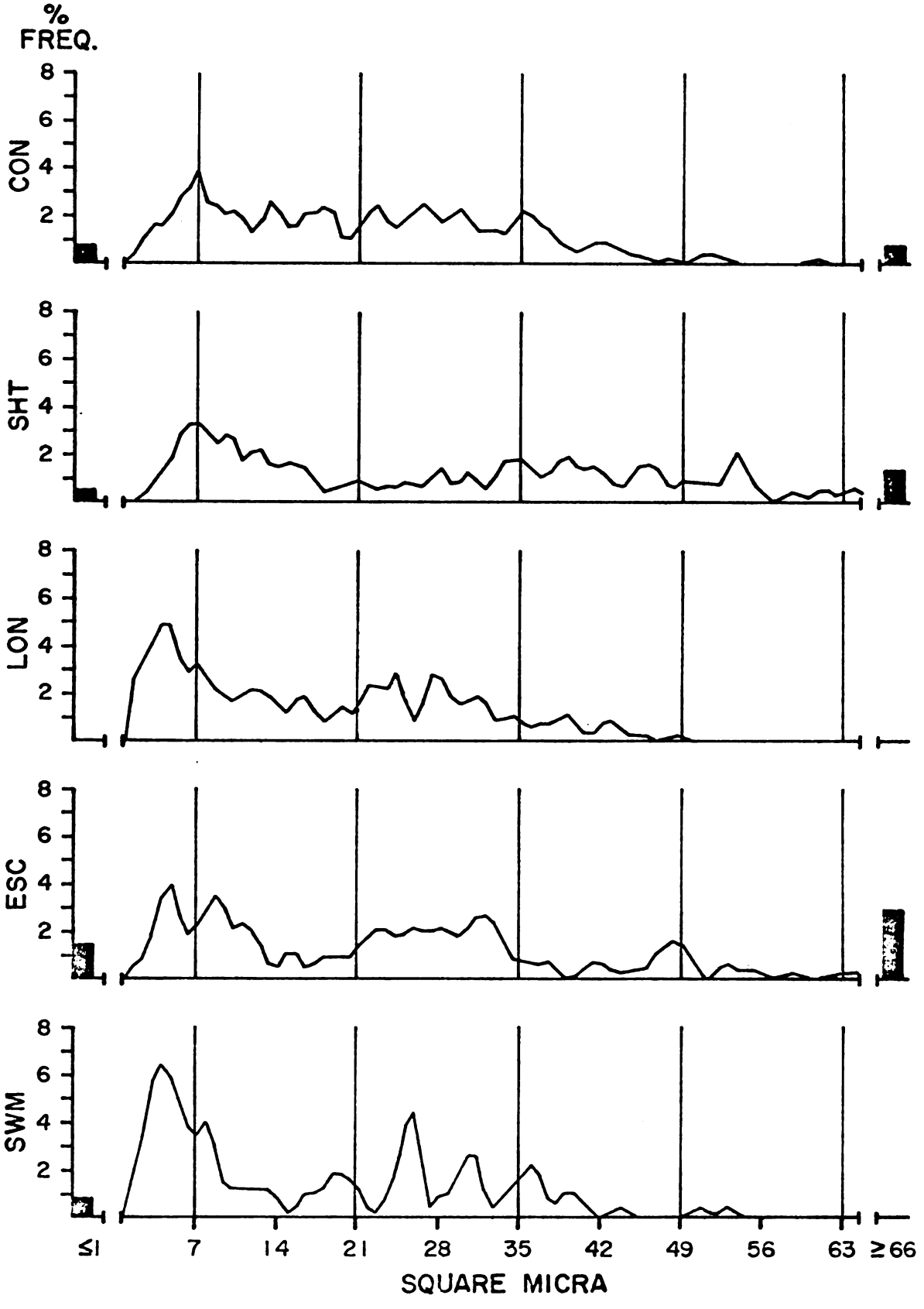
Total Area by Percent Frequency for the Electrical Stimulus Control Experimental Treatment across Duration

Figure 17



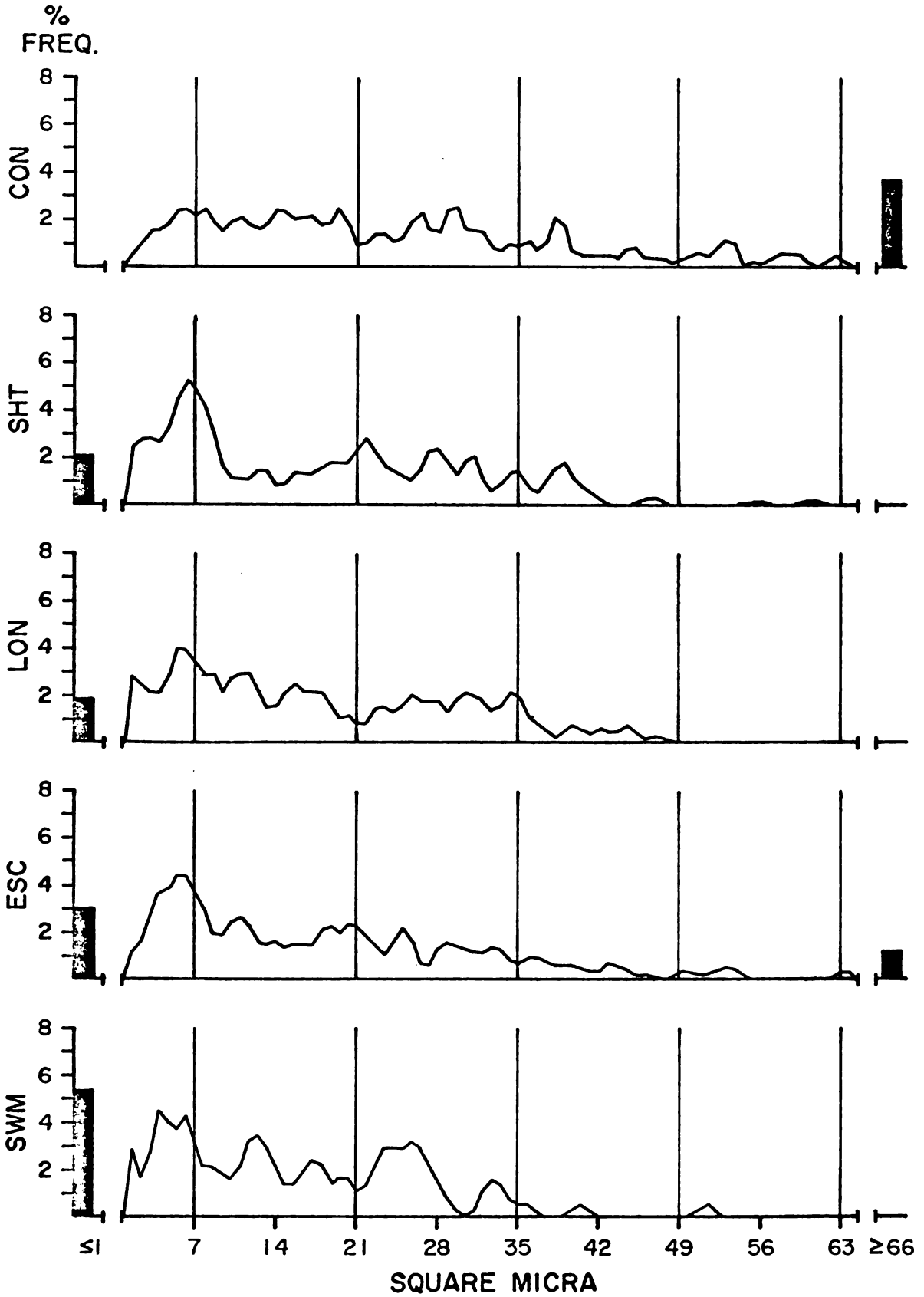
Total Area by Percent Frequency for the Swim Experimental Treatment across Duration

Figure 18



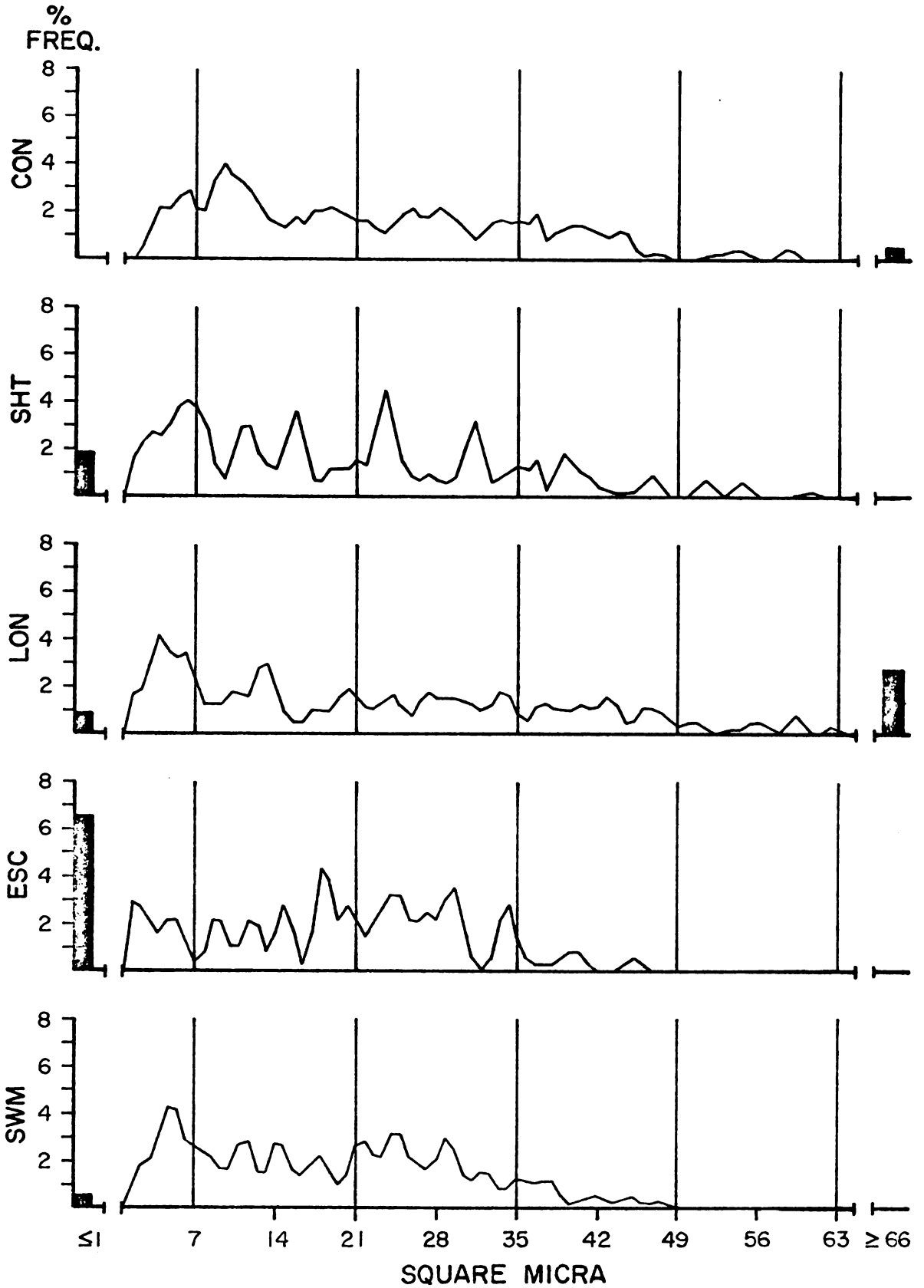
Myelin Area by Percent Frequency for each Experimental Treatment at Four Week Duration

Figure 19



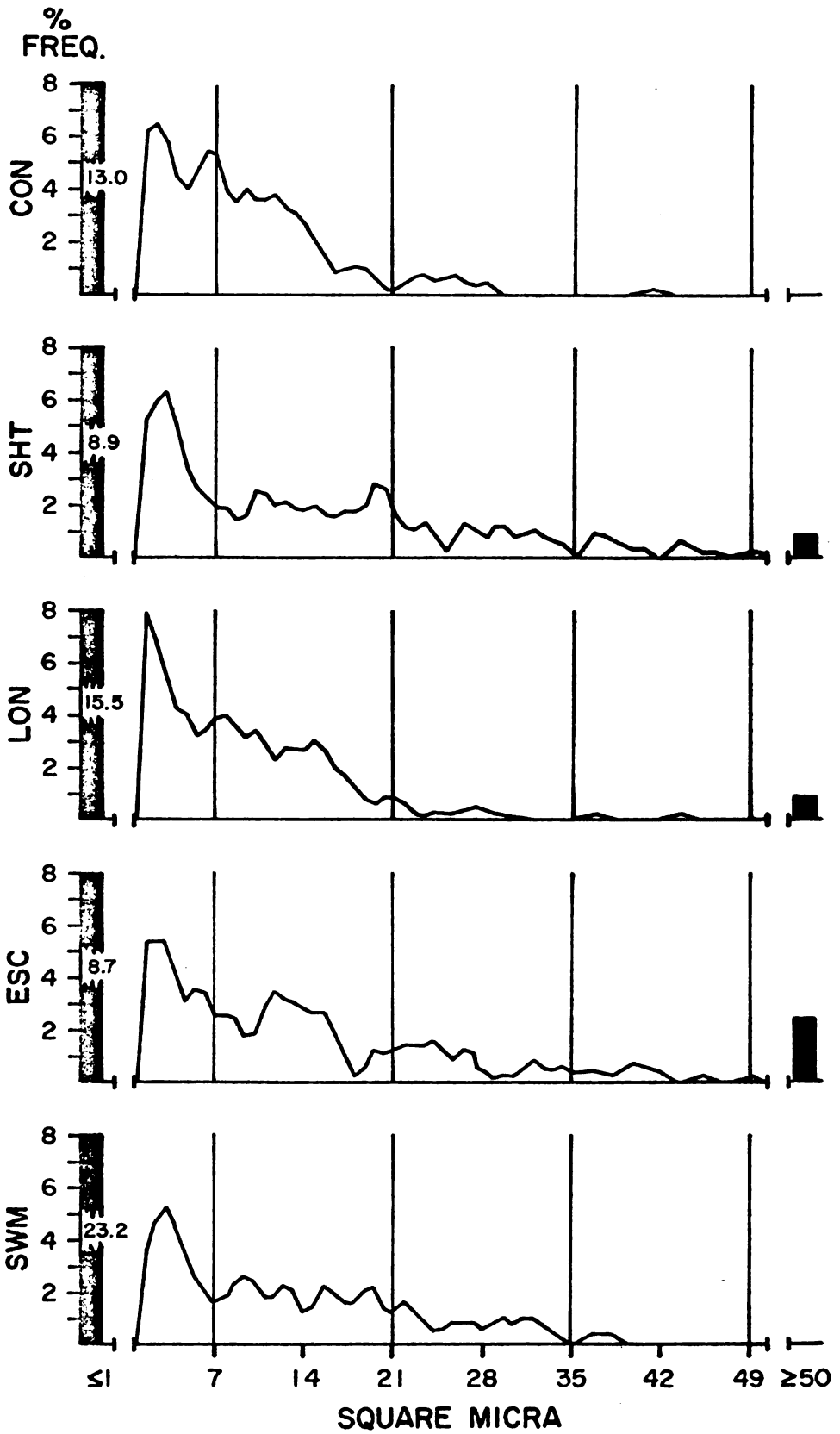
Myelin Area by Percent Frequency for each Experimental Treatment at Eight Week Duration

Figure 20



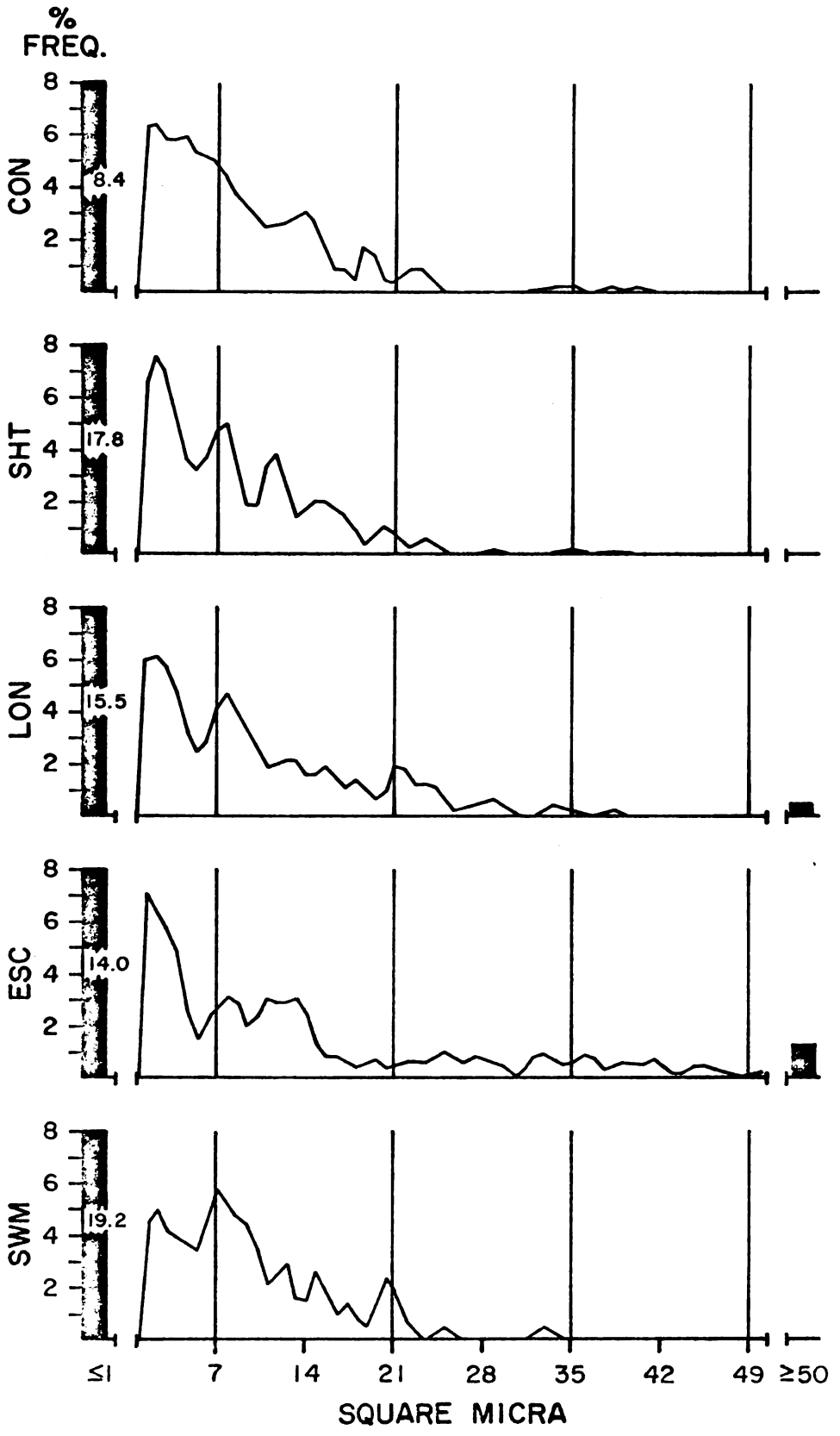
Myelin Area by Percent Frequency for each Experimental Treatment at Twelve Week Duration

Figure 21



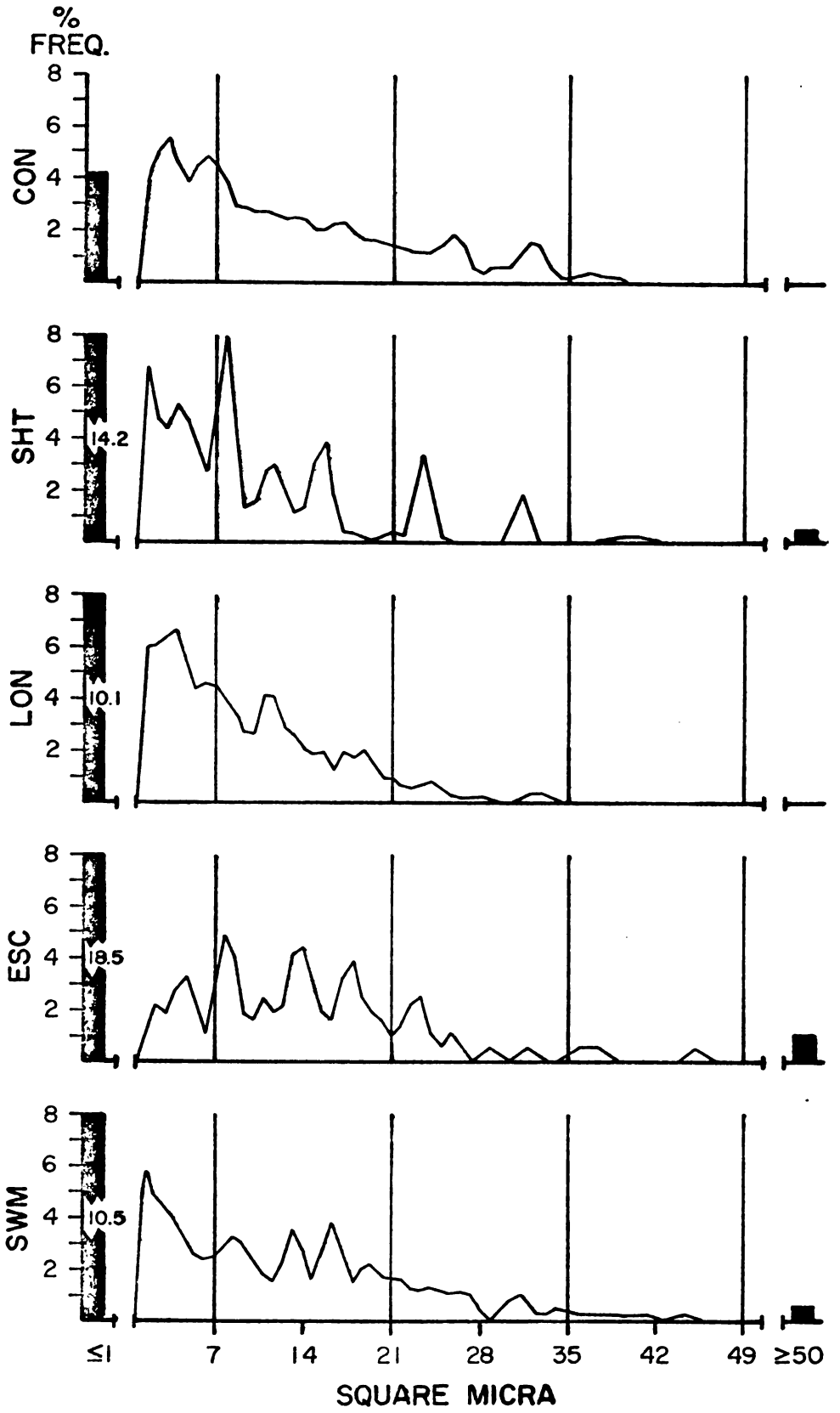
Axon Area by Percent Frequency for each Experimental Treatment at Four Week Duration

Figure 22



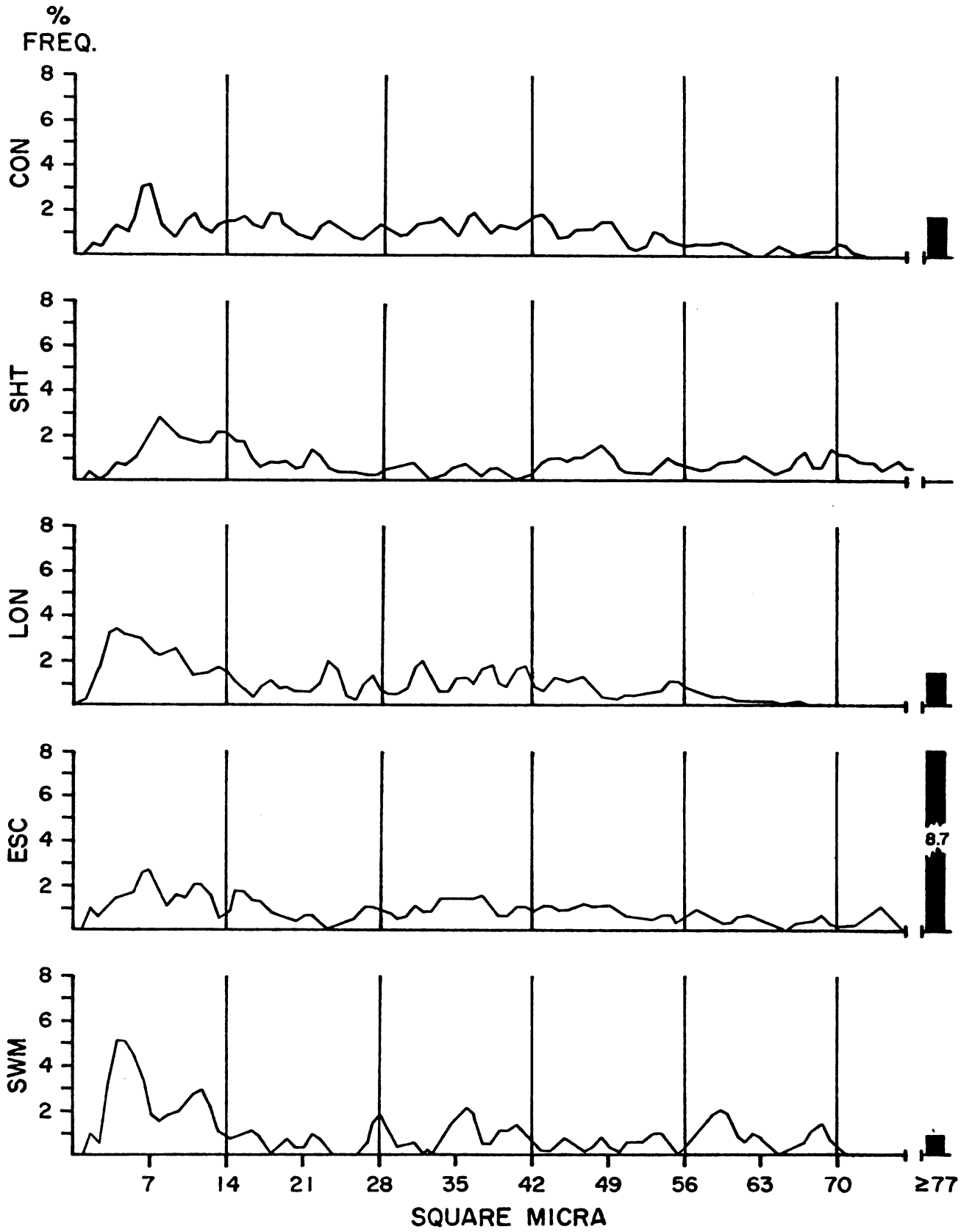
Axon Area by Percent Frequency for each Experimental Treatment at Eight Week Duration

Figure 23



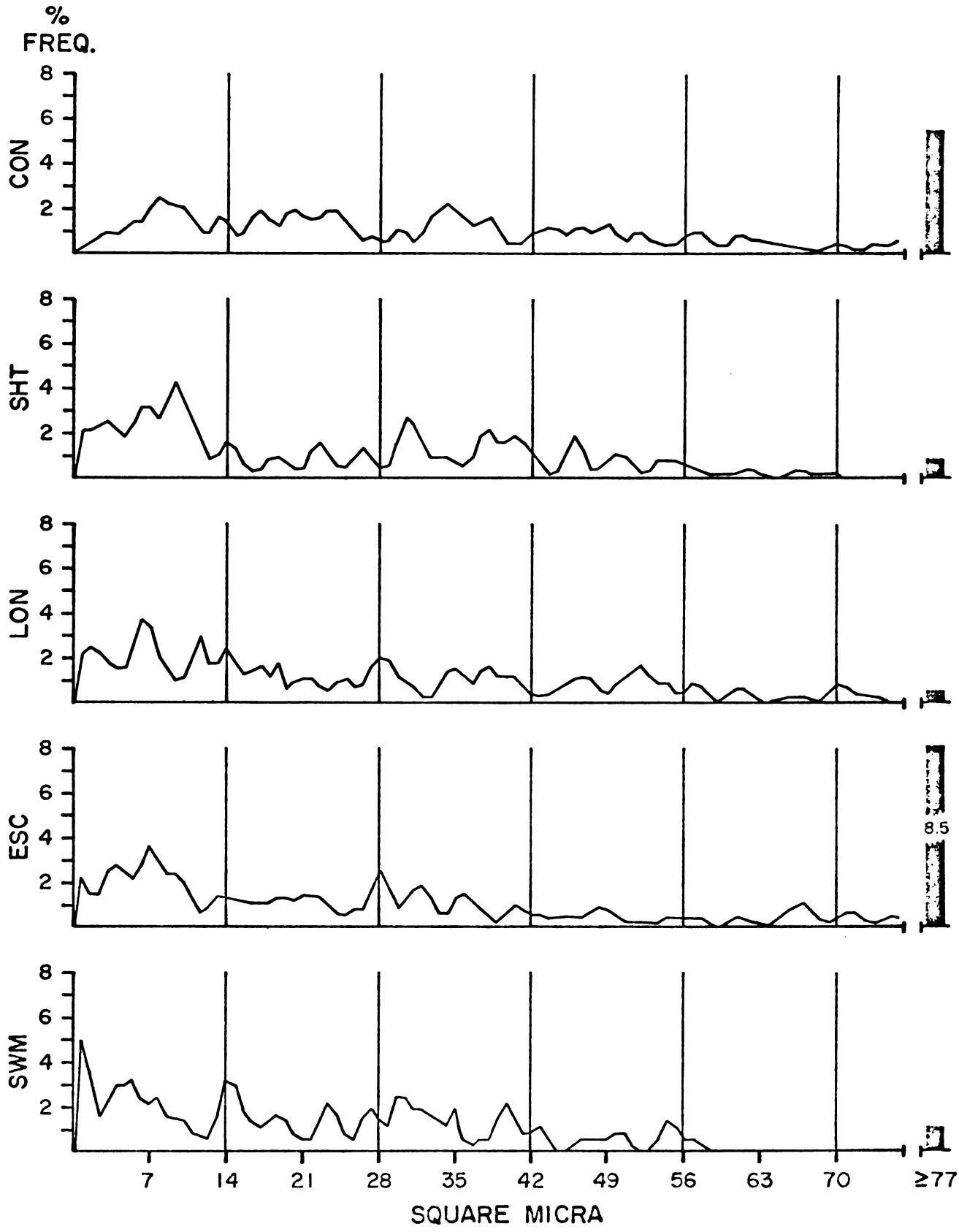
Axon Area by Percent Frequency for each Experimental Treatment at Twelve Week Duration

Figure 24



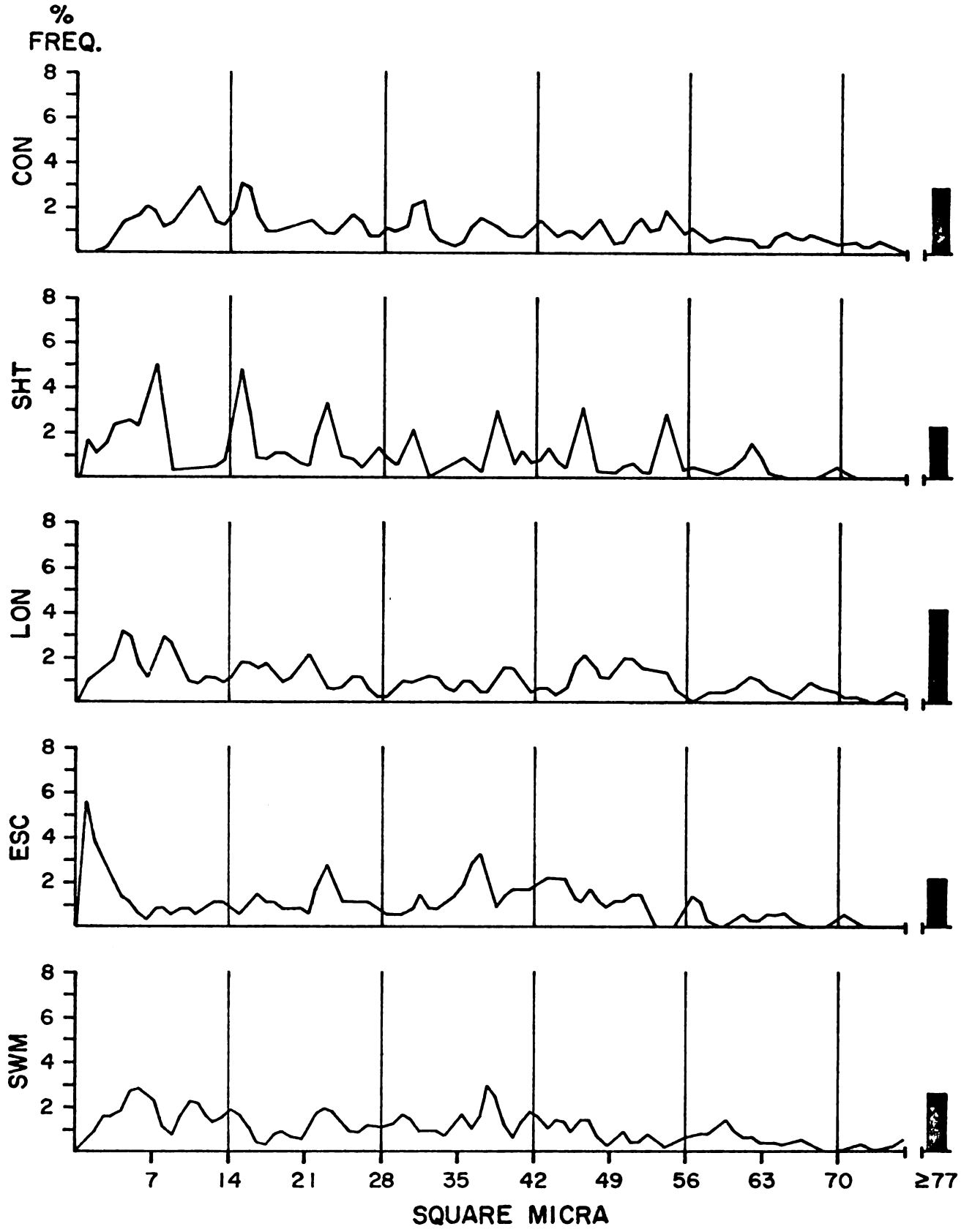
Total Area by Percent Frequency for each
Experimental Treatment at Four Week Duration

Figure 25



Total Area by Percent Frequency for each
Experimental Treatment at Eight Week Duration

Figure 26



Total Area by Percent Frequency for each Experimental Treatment at Twelve Week Duration

Figure 27

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of the present investigation was to determine the effects of four durations of five specific levels of physical activity on the population of myelinated fibers in the soleus nerve of the normal, male albino rat.

Eighty animals were brought into the laboratory and assigned randomly to seven treatment groups. Due to difficulties in techniques, only five treatment groups were utilized in this study: CON, SHT, LON, ESC and SWM. Animals from each group were sacrificed at zero weeks and then at four, eight and twelve weeks after the onset of training.

The left soleus nerve was surgically removed from sodium pentobarbital anesthetized animals. Upon removal, the nerve was fixed, stained, embedded in paraffin, sectioned and mounted on glass slides within two weeks of sacrifice.

Visual images of the entire transverse section of the nerve were projected onto drawing paper with a microprojector. Myelin, axon, and total areas of each myelinated fiber were measured by polar planimetry. Total fiber counts were recorded during the same process.

Percent frequency graphs were constructed for myelin, axon and total areas across durations and treatments. Visual inspection of the plots revealed patterns of specificity in the data. SHT and ESC animals tended to have a larger percentage of small fibers than did

the CON, LON and SWM groups. The graphs of all experimental groups were notably different from those of the CON group. Chi square analyses run on specific pooled groups substantiated the observed trends.

Conclusions

The results of the study have led to the following conclusions:

1. The morphological characteristics of the rat soleus nerve are altered by chronic physical activity.

2. Anaerobic exercise regimens seem to have more notable effects on myelin, axon and total areas than do aerobic programs.

Recommendations

1. The study should be repeated using electron microscopy to confirm the observed results.

2. Many of the plots across duration revealed shifts towards alternate ends at different durations. An extension of the treatment period to 16 or 20 weeks is needed to determine when the direction of the shifts stabilize.

3. Axoplasmic transport processes should be investigated in an attempt to relate physiological processes with morphological changes.

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APPENDIX A

TRAINING PROGRAMS

TABLE A-I

Standard eight-week, short-duration, high-speed endurance training program for postpubertal and adult male rats in controlled running wheels

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
	5=F	5	2.0	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
2	1=M	6	1.5	00:10	10	28	4	5.0	1.2	2.5	51:40	700	1120
	2=T	7	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	3=W	8	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	4=T	9	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	5=F	10	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
3	1=M	11	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	2=T	12	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	3=W	13	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	4=T	14	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	5=F	15	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
4	1=M	16	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	2=T	17	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	3=W	18	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	4=T	19	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	5=F	20	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
5	1=M	21	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	2=T	22	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	3=W	23	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	4=T	24	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	5=F	25	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
6	1=M	26	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	2=T	27	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	3=W	28	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	4=T	29	2.0	00:10	35	10	5	5.0	1.0	5.0	54:34	625	500
	5=F	30	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
7	1=M	31	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	2=T	32	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	3=W	33	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	4=T	34	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	5=F	35	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
8	1=M	36	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	2=T	37	2.0	00:10	40	6	8	2.5	1.0	5.5	52.10	660	480
	3=W	38	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	4=T	39	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	5=F	40	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSP* and *PER* scores of 75 or higher during the final two weeks. Alterations in the work time, rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard short-duration, high-speed endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
1.5	00:30	30	6	3	5.0	1.0	4.0	26:30	540	540

TABLE A-2

Standard eight-week, long-duration, low-speed endurance training program for postpubertal and adult male rats in controlled running wheels

Wk.	Day of Wk.	Day of Tr.	Acc-eler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repe-ti-tions per Bout	No. of Bouts	Time Bet-ween Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revo-lu-tions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:20	10	30	2	5.0	1.2	1.5	34:40	450	1200
	5=F	5	2.5	00:30	15	20	2	5.0	1.2	1.5	34:30	450	1200
2	1=M	6	2.0	00:40	20	15	2	5.0	1.2	2.0	34:20	600	1200
	2=T	7	2.0	00:50	25	12	2	5.0	1.2	2.0	34:10	600	1200
	3=W	8	1.5	01:00	30	10	2	5.0	1.2	2.0	34:00	600	1200
	4=T	9	1.5	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
	5=F	10	1.0	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
3	1=M	11	1.0	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
	2=T	12	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	3=W	13	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	4=T	14	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	5=F	15	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
4	1=M	16	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	2=T	17	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	3=W	18	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	4=T	19	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	5=F	20	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
5	1=M	21	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	2=T	22	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	3=W	23	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	4=T	24	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	5=F	25	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
6	1=M	26	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	2=T	27	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	3=W	28	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	4=T	29	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	5=F	30	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
7	1=M	31	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	2=T	32	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	3=W	33	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	4=T	34	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	5=F	35	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
8	1=M	36	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	2=T	37	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	3=W	38	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	4=T	39	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	5=F	40	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSF* and *PER* scores of 75 or higher during the final two weeks. Alterations in the work time, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training programs.

Standard long-duration, low-speed endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

Acc-eler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repe-ti-tions per Bout	No. of Bouts	Time Bet-ween Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revo-lu-tions *TER*	Total Work Time (sec) *TWT*
1.0	12:30	0	1	2	2.5	1.0	2.0	27:30	750	1500

TABLE A-3

Standard eight-week, endurance, swimming training program for postpubertal and adult male rats

Wk.	Day of Wk.	Day of Tr.	Per Cent Tail Weight	Expected Swim Time (min) *EST*
1	1=M	1	0	30
	2=T	2	0	40
	3=W	3	C*	50
	4=T	4	C	60
	5=F	5	C	60
2	1=M	6	2	40
	2=T	7	2	40
	3=W	8	2	40
	4=T	9	2	45
	5=F	10	2	50
3	1=M	11	3	30
	2=T	12	3	30
	3=W	13	3	30
	4=T	14	3	35
	5=F	15	3	35
4	1=M	16	3	35
	2=T	17	3	40
	3=W	18	3	40
	4=T	19	3	40
	5=F	20	3	40
5	1=M	21	3	40
	2=T	22	3	45
	3=W	23	3	45
	4=T	24	3	45
	5=F	25	3	45
6	1=M	26	3	45
	2=T	27	3	50
	3=W	28	3	50
	4=T	29	3	50
	5=F	30	3	50
7	1=M	31	3	50
	2=T	32	3	55
	3=W	33	3	55
	4=T	34	3	55
	5=F	35	3	55
8	1=M	36	3	55
	2=T	37	3	60
	3=W	38	3	60
	4=T	39	3	60
	5=F	40	3	60

*C = clothes pin only.

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 90 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PET* scores of 75 or higher during the final two weeks. Alterations in the per cent tail weight or expected swim time can be used to affect changes in those values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a severe, sudden exercise stress upon the animals and will seriously impair the effectiveness of the training program.

Standard endurance swimming maintenance program for postpubertal and adult male rats.

Per Cent Tail Weight	Expected Swim Time (min) *EST*
2	40

APPENDIX B

ENVIRONMENTAL CONDITIONS AND BODY WEIGHT VALUES

Table B-1. Treatment environmental and body weight values for SHT and LON

Variable	N[1]	Mean	Stan. Dev.	Simple Correlations					
				Air Temp.	% Humidity	Bar. Press.	Pre-Treat. Body Wgt.	% Body Wgt. Loss	PER
Air Temp. (°C.)	473	23.6	2.06						
% Humidity	473	43.3	10.85	0.306					
Bar. Press. (mmHg)	473	740.8	4.38	-0.222	-0.247				
Pre-Treat. Body Wgt. (grams)	468	378.1	35.07	-0.165	0.466	-0.041			
% Body Wgt. Loss	468	2.4	1.04	-0.006	0.024	-0.036	0.041		
PER	473	108.5	26.40	0.100	-0.273	-0.006	-0.558	0.116	
PSF	473	88.2	10.36	-0.106	-0.083	-0.015	0.028	0.216	0.498

[1]Total training days for all animals.

Table B-2. Treatment environmental and body weight values for SWM

Variable	N[1]	Mean	Stan. Dev.	Simple Correlations: Water Temp.	Air Temp.	% Humidity	Bar. Press.	Pre-Treat. Body Wgt.
Water Temp. (°C.)	179	31.8	0.42					
Air Temp. (°C.)	179	23.2	2.04	0.065				
% Humidity	179	45.0	7.45	-0.083	-0.051			
Bar. Press. (mmHg)	179	741.4	3.64	0.138	-0.269	-0.004		
Pre-Treat. Body Wgt. (grams)	179	380.3	42.89	-0.232	-0.189	0.402	-0.020	
PET	179	99.8	3.11	-0.030	0.030	-0.141	-0.005	-0.033

[1]Total training days for all animals.