A METHOD FOR THE CONTROLLED MUSCULAR EXERCISE OF LABORATORY RATS

Thesis for the Degree of M. A.

MICHIGAN STATE UNIVERSITY

Kenneth Douglas Coutts

1964

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By

Kenneth Douglas Coutts

AN ABSTRACT OF

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF ARTS

Department of Health, Physical Education and Recreation

1964

Approved: Wayne Van Huss

ABSTRACT

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EXERCISE OF LABORATORY RATS

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It was the purpose of this study to develop a method for the exercise of a specific muscle group in the laboratory rat. The method described in this paper should provide a new approach to the study of the effects of muscular exercise. Previous methods of exercising laboratory animals, such as swimming, treadmill running, and revolving exercise cages, deal with the whole animal. The development of a method for exercising a specific muscle or muscle group will not replace these methods, but will provide the means for studying some of the effects of this specific type of exercise.

A method for controlling the muscular contractions of the animal was the first consideration of this study. Electrical stimulation of the nerve to the muscle or muscle group was considered as one of the best of possible ways to control muscular contractions. Stimulation of the nerve, rather than the muscle, seemed to be a better method due to a better control of permanent electrode placement for use in chronic experiments and it would also be a more natural pathway for stimulating muscular contractions.

The method includes the design of an exercise rack and a means of measuring and recording the work output and force

of the muscular contractions during exercise. The exercise rack is designed primarily as a means of immobilizing and stabilizing the animal while providing freedom of movement for the body part being exercised. There are several methods which can be used to measure the work output and force of muscular contractions, but only two were developed and adapted for use with this method.

The method developed in this study was specifically designed for exercising the gastrocnemius muscle, but other muscles involved in knee flexion and plantar flexion of the foot are activated through stimulation of the sciatic nerve. The general concept involved in this method, however, can be applied to other body movements and muscle groups.

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ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Wayne VanHuss and Dr. William Heusner for their initial idea for this study and their assistance throughout.

I also wish to thank my wife, Nancy, for her help with the illustrations and her moral support which facilitated the completion of this paper.

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CHAPTER I

INTRODUCTION

The method described in this paper was developed to provide a means of studying the effects of muscular exercise on laboratory rats. It is hoped that a method for controlled muscular exercise of laboratory rats will provide a means for studying some of the basic histiological, anatomical, and chemical changes brought about by muscular exercise. In contrast to exercise methods such as swimming, treadmill running, and revolving cage running used in previous studies on laboratory animals, this method deals with the control of the contraction of a specific muscle or muscle group rather than exercise of the whole body.

Since the exercise program in most studies that would use this method will last at least several weeks, the method for controlling the muscular contractions must be suited to keep the animals alive and healthy for this period of time. Electrical stimulation of the sciatic nerve in a rat was thought to be a most satisfactory means of controlling the contraction of the knee flexor muscles because of its simplicity and effectiveness. Nerve stimulation was used rather than direct muscle stimulation since it provides a more normal pathway for eliciting muscular contractions and better control of permanent electrode placement. Implantation of permanent electrodes beneath the skin allows the animal almost complete freedom of movement with only two small insulated conducting wires protruding through the skin for connection to an electrical stimulator. Any electrical stimulator can be used, but a standard physiological stimulator with frequency, duration, and strength of stimulus controls seems best suited for the amount of control necessary in most exercise programs.

Since the animal must be anesthetized during exercise to eliminate voluntary movements, an exercise rack was needed to hold the animal in place yet allowing freedom of movement in the limb being exercised. Quantitative and qualitative methods were also needed for measuring and recording dynamic and static muscular contractions.

The method developed in this study was specifically designed for exercising the gastrocnemius muscle of the laboratory rat, but other muscles involved in knee flexion and plantar flexion of the foot are also stimulated through stimulation of the sciatic nerve.

CHAPTER II

REVIEW OF PERTINENT LITERATURE

It is beyond the scope of this paper to report on all the different types of electrodes, contraction recording devices, anesthetics, and exercise racks which are in the literature. Only a few references were selected which applied specifically to or formed the basis for the method developed in this study.

Electrodes

The electrodes used in this method were adapted from electrodes developed by L. A. Cohen.(3) The electrodes developed by Cohen were developed specifically for peripheral nerve studies and for use in acute experiments.(3) The electrodes can be inserted around the nerve with conducting wires protruding from a closed incision which permits freedom of movement for the animal and minimizes drying and temperature control problems.(3) The electrodes designed by Cohen consist of two tin foil strips of appropriate width and length backed on a piece of Parafilm.(3) The ends of the tin foil strips are then covered with strips of Parafilm with an exposed portion in the middle of each foil strip for contact with the nerve.(3) The insulated conducting wires are attached to the foil strips and can be connected to any type of stimulator or recorder.(3) In placing the electrodes on the nerve, the foil strips are bent around the nerve with the exposed foil in contact with the nerve and melted paraffin is used to seal the electrode around the nerve.(3)

Work Output Recording

Two methods for recording the work output of a flexing leg of laboratory rats were developed by Heron, Hales, and Ingle (6) and Ingle (7).

The method presented by Heron, Hales, and Ingle in 1934, consists of a light wheel attached to the shaft of a Veeder counter.(6) The wheel is rotated by means of a stationary dog on a shaft freely rotating on the shaft of the Veeder counter.(6) When the leg is flexed, a cord from the leg, which is weighted, moves the stationary dog upward by means of a pulley without rotating the wheel or counter.(6) When the leg relaxes, the weight, by means of the pulley, pulls the dog down.(6) The dog engages on the wheel on the way down which activates the counter.(6)

The method presented by Ingle in 1944 is a more compact and sophisticated application of the method presented in 1934.(7) A cord from the leg which is weighted passes around a pulley which is directly connected to the shaft of a Veeder counter by means of a compact one way clutch within the pulley.(7)

Exercise Rack

Ingle also describes and illustrates an exercise rack which was used in the study on work output in rats.(7) The rat was strapped to a board with the hind limbs extending over the edge of the board.(7) The hind limbs were then secured by means of a hemostat attached to the distal end of the tibia and a thread supported from above is sewn into the back of the rat above the hind limbs.(7)

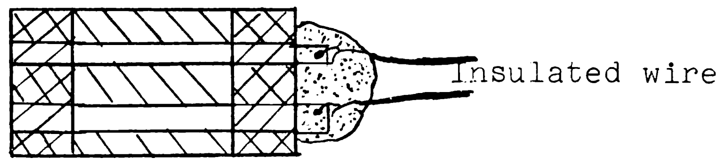
CHAPTER III

METHOD

The main parts of the method developed for controlled muscular contractions in laboratory rats consisted of the construction of the electrodes, the permanent implantation of the electrodes, the construction of an exercise rack, and the development of methods for the quantitative and qualitative measurement of the muscular contractions.

Construction of Electrodes

The electrodes used in this method are similar to those described by Cohen with several adaptations to suit the specific situation.(3) The electrodes were changed from three centimeter long and three millimeter wide strips of tin foil to a length of approximately two centimeters of 0.5 millimeter wide silver ribbon. The change in size and material of the electrodes made soldering of conducting wires impractical. The conducting wires were attached to the electrodes by threading a bare end of the insulated conducting wire through a small hole in the silver ribbon. The exposed wire and any part of the silver ribbon which extend beyond the Parafilm backing is then insulated with paraffin. The rest of the construction is similar to the design described by Cohen.(3)



Scale 1 inch ≅ 1 millimeter

= Bottom layer of Parafilm
= Silver ribbon
= Top layer of Paramilm
= Paraffin

Figure 1. Diagram of Electrodes

<u>Implantation of the Electrodes</u>

The implantation of the electrodes includes the placement of the electrode around the sciatic nerve of the rat with the conducting wires brought up to the back of the neck to prevent interference with the extruding portion of the wires.

After anesthetizing the rat, the initial incision is a vertical cut through the skin, of from five to six centimeters, depending on the size of the rat, starting at a point just posterior and below the greater trochanter of the femur and extending down toward the lateral surface of the knee joint. The underlying fascia is then cut to permit exposure of the hip and upper leg muscles. A two to three centimeter incision is then made by teasing apart the gluteus maximus and biceps femoris muscles. The sciatic nerve is usually found beneath and slightly posterior to the muscle separation.(5) The fascia is then teased away from a section of the nerve, and the electrodes are slipped under and around the nerve and sealed with paraffin. The connecting wires are then brought up to a small skin incision in the back of the neck by means of a probe forced from the hip incision up the back of the rat just beneath the skin to the small incision in the back of the neck. Enough slack should be left in the connecting wires to allow freedom of movement without causing tension on the electrodes, a simple knot is tied in the connecting wires beneath the skin at the neck incision to prevent any pull on the electrode-nerve connection. The lead wires protruding from the back of the neck are sewn into one of the stitches when suturing the neck incision, and a knot is tied in the lead wires to prevent them from slipping beneath the skin. In sewing up the hip incision, a few stitches should be taken to reclose the muscle separation which will help to hold the electrodes in place.

At least two weeks should be allowed for the rat to reocver from the surgery. The rat should be permitted free use of the leg during recovery by placement in an exercise cage to prevent contractions in the leg.

Exercise Rack

The exercise rack designed in this study was developed to permit chronic exercise experiments on laboratory rats.

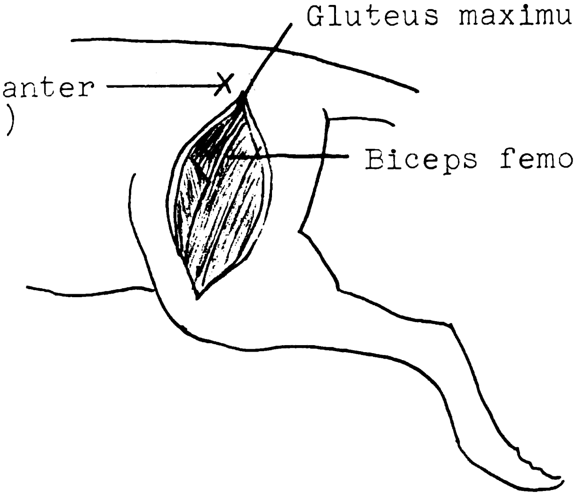


Figure 2. Skin Incision

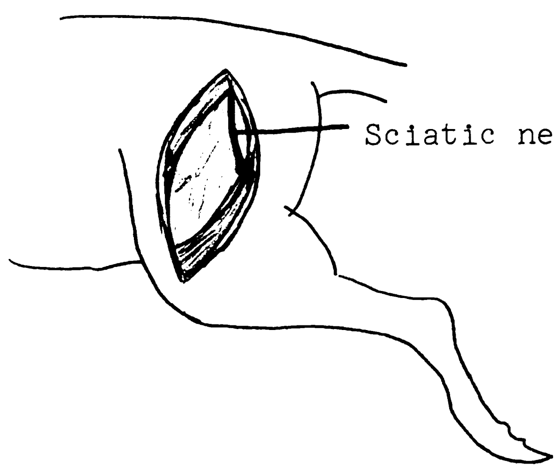


Figure 3. Muscle Incision

The exercise rack was designed to immobilize the rat except for the body part being exercised, and to provide a stabilized base for the muscular contractions.

The exercise rack consists of a raised or suspended length of one inch by two inch board. The length of the board should be at least twice the length of the rat measured from the nose to the proximal end of the tail to permit use of the same rack for stimulation of either leg, and the width should give a stable base while allowing relative freedom of movement of the knee joint with the rat stradling the width of the board. With the rat astride the board, it can be strapped in by criss-crossing string over the rat and attached to hooks placed in the side of the board.(7)

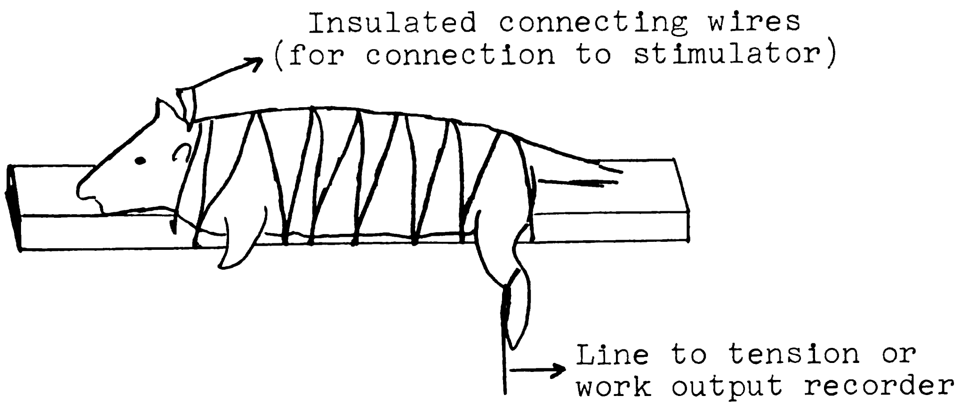


Figure 4. Diagram of Exercise Rack

Measurement of Exercise

Static and dynamic muscular contractions are the two major types of exercise for which this method was developed. A static muscular contraction being defined as a contraction of the muscle without movement of the associated body part, which means that the force of contraction is opposed by a force equal to or greater than the force of contraction. A dynamic muscular contraction is defined as a contraction of the muscle with a movement of the associated body part, which means that the force of contraction is greater than any opposing force or resistance.

Static training or measurement of a static contraction can be accomplished by tying down the foot of the rat with the knee set at any desired angle of contraction. A strain gauge can then be placed in the circuit with adequate amplification to obtain a measure of the force of a contraction.

Dynamic training needs a unit of measure which is different from the static measure since a dynamic contraction involves the application of a force through a given range of motion rather than at a given angle. The most suitable unit of measure seems to be work output, force times distance, which Ingle used in his studies on work output of rats.(7) Heron, Hales, and Ingle and Ingle developed two methods for recording the work output of the dynamic contractions of the legs of laboratory rats.(6, 7) Either of these techniques would be suitable in almost any experiment using this method of exercising rats. Other methods of measuring work output can be developed to suit almost any defined limits of measurement, remembering that force and distance are the two variables necessary to compute work output.

CHAPTER IV

SUMMARY

The development of a method for the controlled muscular exercise of laboratory rats was primarily a process of selecting electrodes which could be used in chronic stimulation exercise experiments and combining it with an adaptation of Ingle's method for studying work output in rats.(3, 7) Ingle's work provided a sound basis for the development of this method for the controlled muscular exercise of rats.(7) Certain adaptations to Ingle's methods were necessary, however, in order to develop a method more suitable for long range experiments. The implantation of permanent electrodes on the sciatic nerve, which permit intermittent exercise bouts rather than a single measure of work output, is the primary change from Ingle's method of direct muscle stimulation.(7) A slight change in the exercise rack used by Ingle was also necessary for use in long range experiments.(7) Ingle's methods for measuring and recording work output plus any method for measuring muscle tension provide a means of obtaining quantitative and qualitative measures of both static and dynamic muscle contractions.(6, 7)

At present, a pilot study is being started in an attempt to perfect this method for exercising rats. The primary aims of this pilot study will be to study the long range effects of the anesthetic and stimulation, and to adapt the method for use with a large number of animals in an experimental situation.

Future plans for the use of this method in exercise studies include several studies on the basic physiological adaptations which are made to the stress of muscular exercise. Tissue analysis, muscle capillarization, serum cholestrol, RNA/DNA ratios, organ weights, and carcass analysis are some of the measurements which will be taken in an attempt to evaluate the effects of muscular exercise on the body. It will also be possible to study the relative effects of different types and intensities of muscular exercise.

This method for the controlled muscular exercise of laboratory rats is not a finished product, but rather a small, initial step toward a better understanding of muscular exercise.

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