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This investigation was designed to determine the influence of paresis on the rate of fatigue induction in mature Sprague-Dawley rats forced to exercise by swimming. A pilot study provided information on the swimming characteristics of the rats, the selection and suitable injection method of a paresis-inducing drug, and the recovery time necessary for the reinstatement of pre-swim and pre-drug conditions.

The experimental procedure employed the use of thirty littermate rats in a one group design with each animal serving as its own control. Each rat was subjected to the following regimen:

1. A marathon swim for endurance and total time to fatigue.

2. A swim time t_o under conditions of paresis

followed by a swim time t_x.

3. A swim time t_o under normal conditions followed by a swim time t_y.

4. A period of time t_o under conditions of paresis, but without the swimming, followed by a swim time t_z.

5. A marathon swim for endurance and total time to fatigue.

A seventy-two hour recovery period was allowed between each procedure. The influence of the paresis was obtained by comparing the difference between the average of the two marathon swims and the swim time t_x with the difference between the average of the marathon swims and the swim times t_y plus t_z.

The student t was used for the test of significance. The results indicated that the paresis was effective in fatigue rate. The difference was found to be significant at the .001 level and the null hypothesis was rejected. In addition a comparison of the pre- and post-experimental marathon swims indicated that no training developed incident to the swimming undertaken during the course of the experimental procedure.

The influence of paresis on the fatigability examined in this study suggests the involvement of central neuron pathways. The influence of peripheral factors such

as lactacidemia or failure in the contractile mechanism were obviated as causal influences through the induction of the paresis. Synaptic fatigue or variance between the neural activity required and the movement perceived during the performance of the exercise are offered as attractive variables which might be influencial in producing the fatigue developed concomitant with the paretic movements.

Further investigation into the mechanisms involved and the influence of various anti-fatigue drugs is recommended.

A COMPARISON OF FATIGUE INDUCTION UNDER PARETIC AND NON-PARETIC CONDITIONS

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TABLE OF CONTENTS

I. Introduction 1
Statement of the Problem 2
Hypothesis 3
Delimitation of the Problem 3
Justification of the Study 3
Definition of Terms 6
Summary 7

II. Review of Relevant Literature 8
Physiological Mechanisms in Fatigue 8
Neuromuscular Blocking Agents 14
Competitive Inhibitors 16
Depolarizing Agents 19
Studies Related to Endurance Exercises in Rats 20
Summary 23

III. Methods and Procedure 25
Pilot Study 25
Experimental Procedure 30

IV. Data Presentation and Discussion 36
Discussion 40

V. Conclusions and Recommendations 44
Bibliography 49
Appendix 54

A COMPARISON OF FATIGUE INDUCTION UNDER PARETIC AND NON-PARETIC CONDITIONS

CHAPTER I

INTRODUCTION

The apparently universal experience of fatigue has remained an enigma despite concerted efforts by many researchers to elicit its nature. Part of the problem in identification may be ascribed to the frequently ambiguous use of the term fatigue. Fatigue is often the only complaint in common among those who do nothing and those who overdo everything. Thus, at the outset, a student of fatigue is plagued with the problem of studying something which, in its subjective experience, can be elicited by two apparently antonymous causes. This paradoxical state of affairs is nicely exemplified in the disease myasthenia gravis as described by Emerson and Bragdon (14, p. 708).

> Myasthenia gravis is a chronic disease of unknown etiology, affecting young adults particularly. Its one symptom is great muscular fatigue, quickly produced by repeated movements, which soon disappears following rest. Patients with this disease tire on such slight exertion as combing the hair, chewing and talking, and quickly must stop for rest...
>
> The treatment of myasthenia gravis during its acute stages includes rest in bed and, later, the limitation

of all unnecessary efforts.

The patient experiences fatigue from doing very little, while at the same time the treatment program suggests that he has done too much. The fatigue is genuine in both the behavioral and the subjective sense. That is, movement can be performed only with difficulty and the subsequent tiredness experienced by the individual is profound. Although the decrement in performance has been extensively studied, the asthenia has received little attention. An analysis of this disease suggests that feelings of fatigue can be developed in the absence of significant muscle activity despite a genuine attempt to perform.

Statement of the Problem

The purpose of the present study was to investigate the fatigue produced by maximum effort with a concomitant reduction of movement by the use of a neuromuscular depolarizing agent. More particularly, the problem in this investigation was to determine whether neural activity in the absence of a proportional amount of muscle contraction will affect the fatigue rate in adult rats.

Sub-problems considered were: (1) selection of a suitable motor end plate depolarizing agent; (2) selection

of a suitable measure of fatigue; and (3) an analysis and evaluation of the results.

Hypothesis

A null hypothesis used for this study proposed that there would be no difference in the time required to reach the fatigue point under paretic and non-paretic swimming conditions.

Delimitation of the Problem

This study involved the development of fatigue through the execution of whole body movements in untrained rats. Swimming was used to induce fatigue and paretic conditions were established with but one depolarizing agent. The investigation made use of 30 mature rats of the Sprague-Dawley strain matched for swimming performance and tested between June 1 and September 1, 1970.

Justification of the Study

The nature and cause of fatigue is of universal interest. Though modern man experiences fewer challenges to his physical endurance than was typical of his more agrarian predecessors, he frequently complains of an

exhaustion which may render him practically, though temporarily, inert. Though attempts have been made to explain away such exhaustion on the basis of boredom or stress, the physiological nature of the problem is hardly elucidated by such rhetoric.

The nature of the times requires that a man function at a high level of efficiency. His mobility enables him to be in numerous circumstances over short periods of time thus broadening his potential as an achieving individual in many spheres of personal and inter-personal endeavor. The limitation of this potential is too frequently that subjective experience called fatigue. But the safe extension or elimination of the fatigue threshold must be based upon discernment and understanding relative to the nature and cause of the phenomenon.

The need for better understanding is also indicated by the number of disruptive physiological and psychological states which implicate fatigue as either cause or effect. For example, Selye (47, p. 234-274) following extensive investigation of hormonal defenses mediated by the autonomic nervous system, described extreme fatigue as the "stage of exhaustion" in which further exposure to stress leads to disintegration and death. Fatigue may also constitute a

sufficient insult to induce a variety of communicable diseases. Burrows (7, p. 325) indicates the existence of clinical and some experimental evidence to suggest fatigue as a predisposing factor to infection. Further, Coleman (9, p. 146) suggests a variety of biological conditions, including fatigue, which may predispose one to, or precipitate, psychopathology.

Despite the apparent need for better understanding of the fatigue phenomenon, insufficient attention has been given to the mechanisms of fatigue induced by whole body behavior with a concomitant attempt to examine the possible neural contributions. More information is required to answer such questions as:

1. Why does work decrement sometimes occur in the absence of significant energy expenditure?

2. Do both psychological and physiological fatigue share common causal factors in the central nervous system?

3. What is the explanation for fatigue in certain neurological disorders involving little or no concomitant physical activity?

4. Is fatigue, in the absence of energy expenditure, due to the same mechanism in all instances?

5. How might a fatigue due to central nervous

system factors best be prevented or alleviated?

The answers to these questions await further investigation. The author has elected to continue the search for answers by studying the nature of the fatigue mechanism.

Definition of Terms

One of the difficulties encountered in the examination of fatigue is the ambiguity of the terms involved. The following will represent the author's interpretations of the terms as they pertain to this study.

1. Fatigue: The state or condition of an organ, tissue, or organism in which its response to stimulation is reduced.

2. Fatigue Point: The point in time when a rat, under conditions of a forced swim, cannot remain at the surface of the water and has been below the surface for a period of 20 seconds.

3. Fatigue time: The period of time between the beginning of a swim test and the fatigue point.

4. Exhaustion: An apparent weakness or extreme fatigue with an accompanying inability to respond to stimuli.

5. Recovery Period: A period of 72 hours between

testing procedures during which the animal was allowed to return to the non-fatigue state.

6. Motor Paralysis: The temporary loss of function of skeletal muscle due to the use of a neuromuscular depolarizing agent.

7. Paresis: A degree of paralysis in which some muscular activity is still possible.

8. Pilot Study: A preliminary experiment conducted to establish the suitability of drugs, dosages and experimental conditions.

Summary

There is a need for better understanding of the fatigue phenomenon. Little is known about the possible contribution of neural factors when fatigue is produced as the result of whole body or skilled movements. In this study an attempt was made to determine the possible influence of extensive neural effort on the development of fatigue in the absence of the usual accompanying muscular activity.

CHAPTER II

REVIEW OF RELEVANT LITERATURE

The purpose of this study was to determine whether the rate of fatigue induction in paretic rats is the same as in normal rats. A review of the literature related to this problem is contained in this chapter. Section one presents studies concerned with the physiological mechanisms operative in fatigue. The second section is devoted to research on specific drugs which are capable of producing the paretic effects necessary in the present study. The third portion is a survey of research related to fatigue production in rats using a whole body exercise.

Physiological Mechanisms in Fatigue

Early studies into physiological fatigue have used primarily *in vitro* preparations such as a muscle-nerve preparation of the gastrocnemius. These studies have suggested two possible causes of the progressive decrease in muscular response:

1. transmission fatigue, wherein there is a failure in the transmission of the nerve impulse to the muscle fiber, or

2. contraction fatigue, wherein the muscle fiber fails to respond to motor end plate potentials (40, p. 183). The research suggests that transmission fatigue may be produced by prolonged stimulation of the motor nerve at high frequency (e.g. 60 impulses per second for one hour). Subsequent galvanic stimulation of the muscle demonstrates that there is no loss in contractile capacity even though the response to nerve stimulation may be considerably reduced. Contraction fatigue occurs with less frequent stimulation. Contraction time lengthens and relaxation becomes slower and less complete as fatigue progresses (24, p. 803).

Merton (34, p. 563) suggests that fatigue under normal exercise conditions is peripheral, that is, a contraction fatigue. He compared the decrease in strength of muscle contraction produced by voluntary efforts with that produced by electrical shock. Using the adductor pollicis muscle with a surface pickup for EMG (electromyograph) recordings, no difference was found in the rate of strength decrease between the two procedures and the EMG was not altered during the course of either the voluntary or shock test. This <u>in vivo</u> study strongly suggests that physiological fatigue in the intact organism is due to factors

incident to the contractile process and is not the result of a breakdown in neural or myoneural transmission. Merton (33, p. 221) reaffirmed his conclusions in 1956 and further suggested that evidence to the contrary was based on inadequately controlled studies.

The importance of metabolic factors in fatigue has been known for some time. Contemporary thinking on this subject is summarized by Best and Taylor (4, p. 879) who indicate that metabolic processes incident to exercise establish the limit of physical exertion as the result of lactacidemia and "oxygen debt". When exercise is strenuous or prolonged at a level which promotes anaerobic glycolysis there is a concommitant rise in lactic acid and a failure of the contractile mechanism as ATP production diminishes. That lactacidemia is a major factor in fatigue is also supported by the findings of Myers (37, p. 57-58) who established that muscles rendered ischemic by the use of a strangulating cuff or tourniquet rapidly become fatigued but are restored to normal strength upon the release of the tourniquet and re-establishment of normal circulation.

A few early studies were conducted in which exercise was performed in the absence of conditions producing either transmission or contraction fatigue (38, p. 214; 27, p. 689).

Lactacidemia did not develop and as a result the usual metabolic complications incident to contraction fatigue did not occur. This condition is referred to as the "steady state" in which aerobic and anerobic processes are balanced (4, p. 879).

Additional investigation into the literature indicated that further elaboration concerning contraction fatigue would be redundant. It seems generally accepted that the major cause of fatigue, under exercise conditions which produce lactacidemia, is a breakdown of muscle metabolism and subsequent contraction fatigue (26, 48, 28). The only apparently recognized exception to this is the development of the steady state wherein lactacidemia fails to develop.

More recent investigation into the nature of fatigue suggests some discrepencies in the earlier studies and thus raises questions as to the validity of the conclusions. Merton (31, p. 564) suggested that his results applied only to unskilled movements of a single muscle and that skillful, repetitive movements for a prolonged time might produce a fatigue that is central in origin. Stevens (43, p. 5P) further states that Merton's conclusions with respect to the EMG may be erroneous. In contrast to Merton's findings, Stevens observed that, when the first dorsal interosseous

is monitered in a procedure similar to that of Merton, there is indeed a decrease in the EMG implying a degree of transmission fatigue. He also suggests that Merton's findings might have been the result of contributions to the EMG made by muscles other than the adductor pollicis and further, that such ambiguity does not exist in monitoring the EMG of the first dorsal interosseous. Evans (15, p. 136) and Bartley (1, p. 67-70), on the basis of their reviews, have suggested that fatigue might be central to the muscle tissue and therefore represent a transmission rather than a contraction fatigue.

Three possible sites of transmission fatigue deserve attention. They are: (1) the neuro-effector junction, (2) the neuron proper, and (3) the central synapse. If transmission fatigue occurs the myoneural junction is an unlikely site. Stimulation at a rate of 150 impulses per second is required to produce fatigue at this location and such a high level of stimulation is improbable under conditions of voluntary motor activity (22, p. 85).

The possibility of fatigue within the neuron has been investigated thoroughly and the results have proved to be negative. The chemical changes occurring in a neuron are minute and even under conditions of rather intense

stimulation there is rapid and complete recovery. A nerve fiber in vivo simply cannot be fatigued under the usual conditions of stimulation (46, p. 207).

Since fatigue at the myoneural junction of the neuron is unlikely, it would seem that any central or transmission fatigue must be a synaptic phenomenon. It is well known that fatigue of synaptic transmission occurs (12, 21, 47). However, there is no information in the literature indicating the relative importance of this phenomenon in the general syndrome of fatigue. Guyton (22, p. 82) states that fatigue, which prevents overexcitation of the nervous system and acts as a protective mechanism against excessive neuronal activity, is an important characteristic of synapses. He further states that this fatigue is incident to the exhaustion of transmitter substance since 10,000 normal transmissions could deplete the stores of transmitter in "only a few seconds." In this connection however, Castillo and Katz (8, p. 574) have shown that in transmission fatigue at myoneural junctions there is a decrease in the release of, but not an actual depletion of, the transmitter substance. This finding could have implications for synaptic transmission as well.

Fatigue is a common occurrence in most spinal cord

reflexes. Fatigue, at the synapse of a reflex involving a receptor pathway A and a motor neuron B, can occur without diminishing the reflex involving receptor pathway C and motor neuron B (46, p. 478). This suggests that synapse A-B is fatigued while synapse C-B is not. Again the possibility of a depletion or failure in release of transmitter substance is certainly attractive. However, there has been some suggestion that reflex fatigue results from a decreased excitability of neurons rather than from a depletion of, or a failure in, the release of transmitter substance (25, p. 538).

Neuromuscular Blocking Agents

The selection of a suitable drug for the induction of motor paralysis involved the consideration of three essential factors. The drug must be reasonably fast acting to promote a short period of recovery from its effects. In addition, the drug must exert minimal influence on the central nervous system such that changes in sensorium due to drug action may be eliminated as a variable. Further, there should be no side effects in other body systems. The search for an appropriate agent was guided by these essentials.

The mechanism of neuromuscular transmission as theorized by Eccles (13) some time ago is generally accepted today. Actylcholine is released from synaptic vessicles as the nerve impulse passes over the end buttons of the lower motor neuron. The action of this mediator on the motor end plate of the muscle cell produces a small electrical charge, the end plate potential. This small potential in turn elicits a propagated action potential which spreads over the muscle ultimately resulting in contraction.

The general concept of neuromuscular blockade as presented by Goth (19, p. 143) suggests that neuromuscular transmission may be interrupted in one of two ways.

1. There may be an interference with normal activity at the presynaptic membrane. Thus the synthesis, storage, or release of acetylcholine may be altered.

2. There may be an interference with normal activity at the postsynaptic membrane. Thus the action of acetylcholine on the motor end plate receptor may be blocked by a competitive inhibitor or rendered ineffective as the result of a long lasting depolarization of the motor end plate. Since it was the purpose of this study to investigate the induction of fatigue under neural conditions

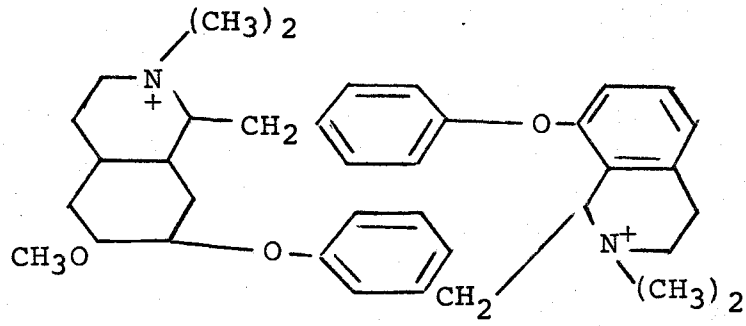
approximating normalcy the author elected to avoid those drugs which could alter normal neuronal activity (i.e., synthesis storage, and/or release of acetylcholine) and selected from those drugs acting only on the effector.

Perhaps the earliest known drug producing neuromuscular blockade is curare. The classic studies by Bernard are cited as the earliest examples of scientific work in pharmacology (19, p. 144). The nature, history and clinical uses of d-tubocurarine, the active principle in curare, have been very thoroughly reviewed by McIntyre (32). The introduction of this purified extract into the armamentarium of anesthesiology spurred efforts to develop synthetic curariform drugs. These efforts have led to the production of several neuromuscular blocking agents which, in addition to curare, were considered for use in this study. These compounds are gallamine triethiodide (Flaxedil), benzoquinonium chloride (Mytolon), decamethonium bromide (Syncurine), and succinylcholine chloride (Anectin) (18, p. 611). Though all of these drugs produce neuromuscular blockade their modes of action fall into two categories; (1) competitive inhibition and (2) agents producing depolarization.

Competitive Inhibitors: These drugs prevent the

access of acetylcholine to the receptor on the motor end plate.

Curare - The active ingredient in curare, d-tubocurarine, has the following structural formula. (6, p. 495)

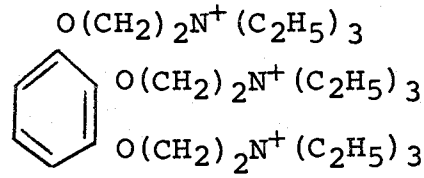


The specific action of the drug seems related to the presence and location of the two quaternary ammonium groups on the molecule (5, p. 541). This observation has been the guiding principle in the manufacture of synthetic curariform drugs.

Curare competes with acetylcholine for the active site on the receptor of the motor end plate. (19, p. 146; 6, p. 494) The release of acetylcholine occurs due to lower motor neuron activity, but the usual subsequent depolarization of the motor end plate fails and flacidity results. In addition to the myoneural activity, other less desirable actions have been noted which suggest that the drug may not be suitable for this study. McIntyre (32, p. 174) noted the electroencephalographic changes elicited by

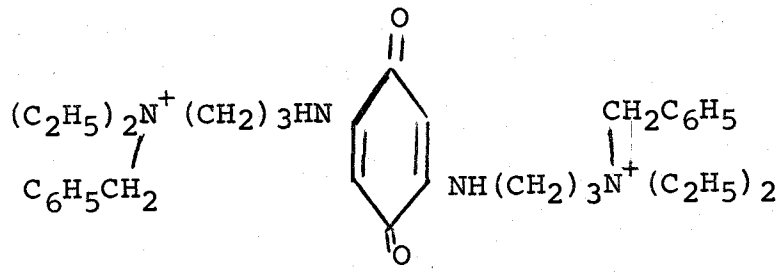
curare and further suggested sensorial changes on rapid injection (32, p. 179). Changes in blood pressure have been noted (10, p. 263), histamine release apparently occurs (35, p. 146), and a residual weakness may persist for thirty minutes or longer (19, p. 145).

Gallamine triethiodide The formula for this compound is as follows (6, p. 498):



The action of this drug is identical to that of curare but with a prolonged effect (6, p. 498) and changes in heart action (39, p. 329).

Benzoquinonium Chloride (6, p. 501)



This compound, in addition to producing a neuromuscular block after the manner of curare, has anticholinesterase activity which produces rather undesirable parasympathomimetic effects such as salivation and bradycardia. However,

some feel that the drug is less offensive than curare in this respect (5, p. 501). Nevertheless, for the purposes of this study, these side effects are undesirable.

Depolarizing agents: These drugs act by depolarizing the motor end plate thus preventing further muscle action after some initial contraction or fasciculation.

Decamethonium bromide - This compound has the following structure (19, p. 147):

$$(CH_3)_3 \equiv N^+ - (CH_2)_{10} - N^+ \equiv (CH_2)_3$$

The primary disadvantage of this drug is its rather prolonged action due to the apparent difficulty with which the drug is hydrolyzed by acetylcholinesterase (6, p. 499). The requirement for a short action period precluded the use of this drug in the present study.

Succinylcholine chloride - This compound has the following structural formula (6, p. 498):

$$(CH_3)_3N^+CH_2CH_2O_2CCH_2CH_2CO_2CH_2CH_2N^+(CH_3)_3$$

This drug, like decamethonium, acts by depolarizing the motor end plate but, unlike decamethonium, the drug is readily hydrolyzed by cholinesterase (16, p. 11). Thus the drug is short acting. An additional advantage is the absence of any side effects other than an occassional apnea

which is avoidable under carefully controlled conditions (19, p. 148). In addition, (in contrast to tubocurarine, gallamine triethiodide, and benzoquinonium) there are no observable effects on the central nervous system with this drug (12, p. 214). Commensurate with these findings, and subsequent experience by the author, this compound was selected as the drug most appropriate for the purposes of this study.

Perhaps it should be noted that anticholinesterases are a conceivable blocking agent. These drugs would block the action of cholinesterase, thus giving rise to an accumulation of acetylcholine, prolonged depolarization, and subsequent flacidity. However, these drugs are parasympathomimetic and are thus undesirable for the present study (42, p. 444).

Studies Related to Endurance Exercises in Rats

This investigation required the production of fatigue by some objectively measurable procedure which would evince any differences in the time required to develop fatigue under normal and paretic conditions. To more nearly insure the development of true fatigue, even to the point of exhaustion, the following criteria were

considered:

1. The exercise should involve an unlearned behavioral response such that the animal will be inclined to exercise in response to an appropriate stimulus without a need for prior training. If training is required, the level of learning could influence both the efficiency and the duration of the fatigue producing exercise thus introducing an extraneous variability.

2. The behavior-producing stimulus should elicit an innate motivation to escape even when exhaustion is imminent. This would insure a continuance of the exercise to exhaustion.

3. The exercise should not produce any pain or injury, with the exception of that directly attributable to the fatigue or exhaustion, which might cause the animal to stop the exercise.

These criteria seemed best met by using swimming as the fatiguing exercise. No training is necessary, motivation to avoid drowning appears high, and neither pain nor tissue damage are likely results.

Several objective measures have been utilized to define the fatigue point in swimming (36, 41, 31), and their reliability has been established relative to more

subjective measures such as disorientation (31, p. 1433). In these studies the fatigue point has been variously set at from ten to sixty seconds.

Though swimming meets the above criteria there are certain difficulties which must be taken into consideration. For example, the temperature of the water is a significant stress factor. Griffiths (20, p. 47) and Matoush (29, p. 263) found that rats can swim for hours in water near body temperature. Decreasing or raising the water temperature shortens the swimming time but renders comparison among animals more difficult and introduces hypo- or hyperthermia as an additional stress (44, p. 150; 49, p. 458). Another method utilized to shorten swimming time is the application of weights to the animal on an absolute (41, p. 195) and on a percentage basis (36, p. 478; 31, p. 1432). Although the use of absolute weight reduces swimming time, considerable variability among individuals is introduced. The addition of weights to rats on a percentage basis seems to favor the smaller animal in water near body temperature (31) but there seems to be no advantage when lower water temperatures (24-26^{o} C) are used (3, p. 599). Thus, in the interests of decreasing swimming time and variability, a percentage weight and

reduced water temperature were employed in this study.

In any study requiring several swim tests the influence of training must be evaluated. Results from other investigations are ambiguous. Some have indicated an improved performance after eight or more swim tests (29, p. 263; 50, p. 201) while others have reported no change (2, p. 697) or an actual decrease in performance (45, p. 714). In the present study it was considered desirable to check for a possible training effect during the course of the investigation.

Summary

A review of the literature has failed to produce studies designed to test whether or not the general fatigue syndrome is in part a central nervous system phenomenon. From the investigations cited it is apparent that, *in vivo*, contraction fatigue plays a major role and that myoneural and neuronal transmission fatigue are of no consequence. However, the possible role of transmission fatigue at synapses requires further study.

The present investigation, designed to augment understanding relative to central mechanisms in fatigue, necessitates the use of a neuromuscular blocking agent.

Although several drugs have been produced which will give rise to a neuromuscular blockade, only succinylcholine demonstrates the specificity of action and freedom from side effects required by this study.

The induction of fatigue by measurable methods seems best achieved through the utilization of swimming as the fatiguing exercise. No learning is necessary, motivation seems high, and extraneous pain and injury are absent.

CHAPTER III

METHODS AND PROCEDURE

The purpose of this study was to determine whether or not a difference exists in the fatigue rate between rats forced to exercise under paretic conditions and those exercised under non-paretic conditions. Forty-five adolescent littermate rats of the Sprague-Dawley strain were obtained and grown to maturity on a standardized diet. The male and female rats were separated by sex and housed in pairs in a kennel maintained at a temperature of 72° Farenheit. By the time a mature weight of 250 gms. was reached and the testing was undertaken, the rats had been living in a controlled environment in excess of six weeks.

Pilot Study

A pilot study was effected to determine reference points for the two basic variables employed in the research. It was necessary first to become familiar with the swimming characteristics of the rats. Based on data from the literature (31; 44; 49; 23) it was decided to swim the rats one at a time in a swim tank. The tank consisted of an opaque circular container thirty inches high and fourteen inches

in diameter. The tank was filled to within six inches of the top with tap water. Through periodic additions of warm water to the tank the temperature of the water was maintained at 30° Centigrade. According to the work of Griffiths and Matoush (20, p. 42; 29, p. 263) this temperature exerts a minimum of thermal stress on the animal while at the same time allowing for the heat dissipation necessary to prevent a hyperthermia that would otherwise tend to develop as a result of the swimming.

The rats were subjected to the swimming portion of the pilot study as follows; each rat, weighted after the manner of Hardin (44, p. 370), was placed in the water to swim. Such weighting (three percent of the body weight) will cause a rat to cease swimming as a result of fatigue and not as a result of the weight being in excess of that which the rat can support while swimming. The weights were attached to the rats with a string harness tied about the chest. The weights were fastened below and near the center of the body to produce minimal interference with swimming movements.

Each rat was subjected to a marathon swim which continued until the rat sank below the surface, remaining there for a period of twenty seconds. At the end of the

twenty seconds the time was recorded and designated as the fatigue point. It was noted during the course of the swim that considerable bouyancy was produced by air trapped in the rats' fur. This effect was minimized by rubbing the fur of the rat while in the water thus displacing the air. It was beneficial to allow the water to stand in the tank for an hour (31, p. 1432; 30, p. 52). This allowed excess air to be liberated from the water which in turn reduced the accumulation of bubbles in the fur of the rat.

As predicted by Hardin (23) the marathon swim lasted for less than thirty minutes under these conditions. It was therefore decided that the experimental procedure would entail subjecting the rats to three percent weight, placing them in the water at 30° Centigrade, rubbing the fur for one minute to eliminate trapped air, and allowing them to swim to the fatigue point as described.

The drug factor was the second variable explored by the pilot procedure. Five rats were subjected to injections of succinylcholine to establish the desirable dosage and the most desirable route of administration. Intravenous injection at various dosages and frequencies was attempted but with unsatisfactory results. The onset of drug action occurred too fast and the duration of effect was too short

by this route. Muscle fasciculations and tremors were noted and, if the rat survived, breathing frequently became shallow accompanied by pronounced cyanosis. Apnea occurred at doses necessary to produce a satisfactory paresis causing three of the animals to die in respiratory collapse.

Subsequently the intraperitoneal route was used. Injection of the drug was accomplished by holding the rat loosely in a vertical position, belly exposed, and injecting at an angle to minimize the trauma to abdominal organs and to reduce the likelihood of an injection into the intestinal wall or lumen. By this route of injection the onset of drug action was slower, the breathing more readily controlled, and the duration of effect within more desirable limits. The most satisfactory results were obtained when the succinylcholine, in an aqueous solution of 0.40mgm per cc., was administered at a dosage of 1.30mgms per kilogram of body weight. The dosage was divided into five equal increments administered five minutes apart. This dosage produced a rather flacid animal with shallow, regular breathing but without cyanosis or dyspnea. Five minutes after the last increment was administered the animal was weighted at three percent, placed in the water, rubbed for one minute to remove trapped air, and allowed to swim to the

fatigue point. In this way it was possible to observe the swimming capacity and characteristics of the rat while under the influence of the drug. Under such conditions the animal was able to swim for only a few minutes. The swimming movements were ataxic and tremulous.

In addition to determining the method of injection and the influence on swimming, the drug was tested to ascertain the period of time required for recovery from the paretic effects. For this investigation, the rat was injected with the drug as above and allowed to recover without swimming. After varying periods of time the rat was weighted and placed in the water as before. As indicated in the literature (18, p. 615) the effects of the drug are extremely variable. This fact was amply demonstrated in the length of the recovery period. It was ultimately necessary to establish an arbitrary recovery period of twenty-five minutes after the last increment injection. After this period of time the animals appeared normal in general behavior and their swimming time upon recovery approximated that of the marathon swim.

In the actual experiment each animal served as its own control. Thus, it was necessary to determine how much time would be required for recovery from the test procedures.

After each procedure in the pilot study the rats were allowed to recover for a period of seventy-two hours. This period of time was chosen as a starting point for the convenience it imparted to the scheduling of the various test procedures. It was found that the seventy-two hours allowed sufficient recovery to prepare the rats for a marathon swim comparable to the first swimming experience.

The pilot study provided the following information for use in the experimental procedure:

1. The dosage and method of drug administration, i.e., 1.30 mg of succinylcholine per kilogram of body weight given in five equal increments five minutes apart and injected intraperitoneally.

2. The recovery period required for dissipation of the drug effect, i.e., twenty five minutes from the last increment injection.

3. The recovery period required between procedures, i.e., seventy-two hours between the end of one procedure and the beginning of the next.

Experimental Procedure

The actual experiment incorporated thirty-three rats in a one group design with each rat serving as its own control. Two rats died during the experiment and one refused

to swim. The apparatus consisted of lead weights, a swimming tank with water at 30° Centigrade, string for harnessing the weight to the rats, a stop watch, a centigrade thermometer, succinylcholine in an aqueous solution of 0.40 mg. per cc., a two cc. hypodermic syringe and a supply of twenty-six gauge needles three quarters of an inch in length.

All experimental tests were preceded by a six hour fast during which food, but not water, deprivation was effected. Preliminary to each test procedure the rat was weighed and harnessed about the thorax. Lead weights equivalent to three percent of the rat's body weight were attached to the harness just before placing the rat in the water. On being placed in the water the rat was rubbed for one minute to remove all trapped air from the fur. The rat was then released to swim. The experimental procedure imposed upon each rat was as follows (See Figure 3.1):

Day 1 - The rat was weighed, weighted at three percent, rubbed to remove trapped air, and placed in the water for a marathon swim. When the rat had been below the surface of the water for twenty seconds (the fatigue point) the time of the swim was recorded. This time was designated the fatigue time t_1.

Day 4 - The rat was first weighed and then injected

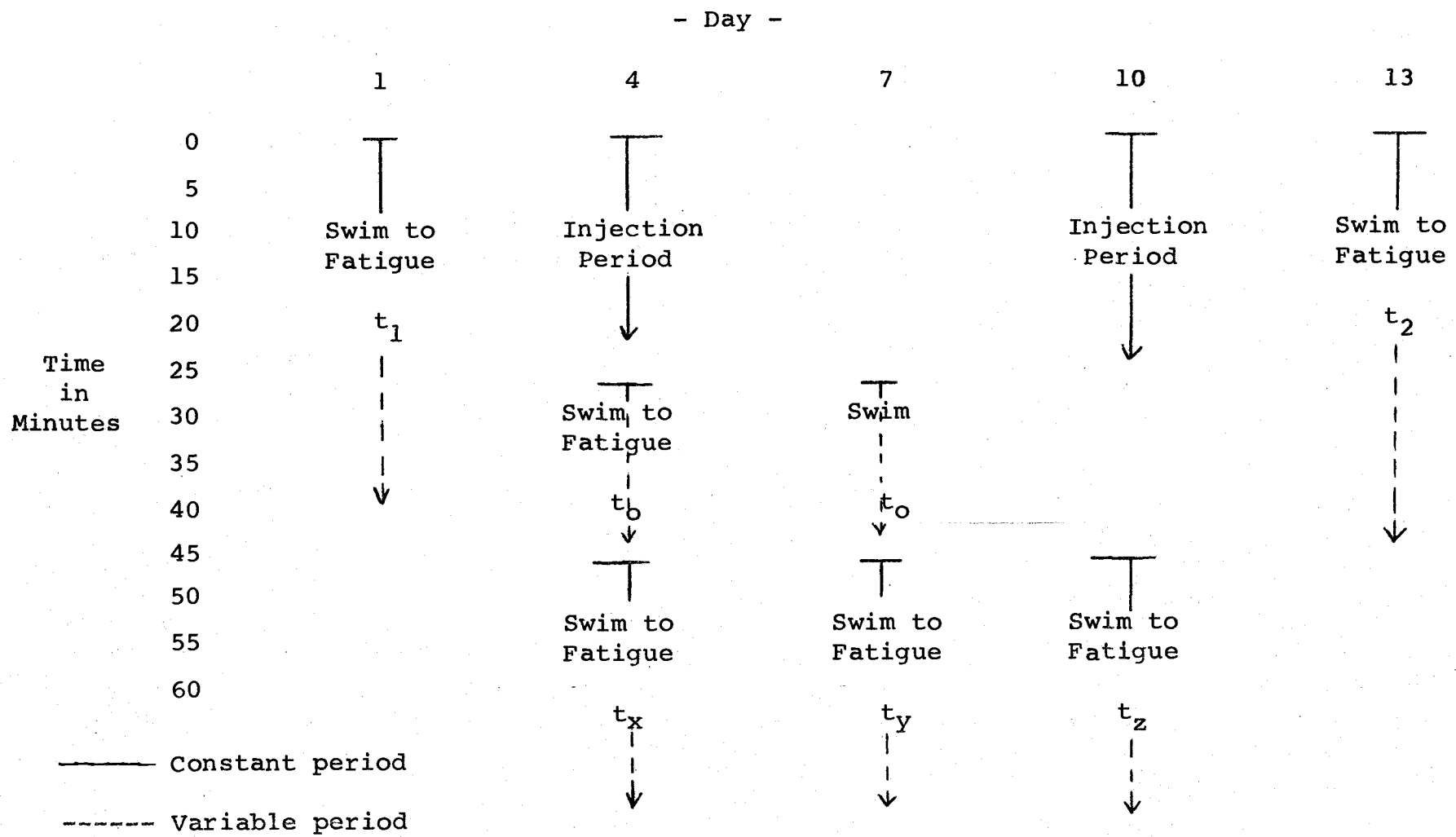


Figure 3.1. Schematic Representation of the Experiment Procedure for Each Rat

with succinylcholine at a dosage of 1.30 mg. per kilogram of body weight. The dosage was divided into five equal increments administered five minutes apart. The rat was then weighted at three percent, placed in the water five minutes after the final increment injection, rubbed for one minute to remove trapped air bubbles, and allowed to swim to the fatigue point as in Day 1. The resultant fatigue time was designated t_o. The rat was then allowed to recover until twenty minutes had elapsed since the beginning of the swim or twenty-five minutes after the final increment injection. The rat was replaced in the water, rubbed as before, and allowed to swim to the fatigue point. This fatigue time was designated t_x.

Day 7 - The rat was weighed, weighted at three percent, placed in the water, rubbed to remove trapped air, and allowed to swim for a time equal to t_o. Recovery was allowed for the remainder of the twenty minute period after which the rat was again placed in the water, rubbed, and allowed to swim to the fatigue point. The fatigue time thus obtained was designated t_y. The Day 7 test was thus identical to the test of Day 4 minus the drug injection.

Day 10 - The animal was given the drug as in Day 4, placed in the water twenty-five minutes after the final

increment injection, rubbed for one minute to remove the trapped air, and allowed to swim to the fatigue point. This fatigue time was designated t_z. The Day 10 test was identical to the Day 4 test minus the swim t_o.

Day 13 - The rat was tested precisely as in Day 1 and the fatigue time was designated t_2. Interim days constituted a recovery period (72 hours), as established by the pilot study, during which the animal was allowed to recuperate from each test procedure.

The paretic swim occurred in the Day 4 test and the resultant fatigue time was designated t_o. The swim performed at the end of this test was influenced by this paretic swim, being shorter than a marathon swim. One or more of the following effects could have produced the influence:

1. The effect of the swim t_o.

2. The effect of the drug induced paresis during the swim t_o.

3. The effects of the drug other than paresis during the swim t_o.

The experimental procedure was designed to evince the possible effect of the drug induced paresis during the swim t_o on the fatigue time t_x.

The mean of the marathon swim fatigue time is given

by the relationship $\frac{t_1+t_2}{2} = \bar{t}$. The expression $t_2 - t_1$ represents any training effect ($t_2 - t_1$ = training effect). In addition the following relationships pertain (refer to Figure 3.1):

1. $\bar{t} - t_y$ = the fatigue time resulting from the effects of the swim t_o (the non-paretic swim effect) = a.

2. $\bar{t} - t_x$ = the fatigue time resulting from the combined effects of the drug induced paresis and the swim t_o (the paretic swim effect) = b.

3. $\bar{t} - t_z$ = the fatigue time resulting from the effects of the drug other than paresis during swim t_o (the drug effect) = c.

To isolate the paretic effect, the influence of the swim t_o and the drug effect must be eliminated. In other words the paretic effect will be found by removing the influence of the swim $t_o(\bar{t} - t_y)$ and the drug effect $(\bar{t} - t_z)$ from the fatigue time produced by the paretic swim $(\bar{t} - t_x)$. Thus, the paretic effect is given by the expression $(\bar{t} - t_x) - [(\bar{t} - t_y) + (\bar{t} - t_z)]$ or $b - (a + c)$. If the combined effect of the swim t_o and drug $(\bar{t} - t_x)$ is equal to the additive effect of the swim $(\bar{t} - t_y)$ plus the drug $(\bar{t} - t_z)$ then $b = a + c$ or $b - (a + c) = 0$ and the paresis is without effect on the fatigue time t_x.

CHAPTER IV

DATA PRESENTATION AND DISCUSSION

This chapter consists of an analysis of the data collected during the course of the experimental procedure and a discussion of the results and their implications. The raw data are contained in Appendix.

A t test was employed to compare the paretic and non-paretic preformances. (Table I presents the results of the analysis.) For each animal the fatigue produced by the paretic swim is represented by the expression $(\bar{t} - t_x)$ and the fatigue produced by the non-paretic swim is given by the expression $[(\bar{t} - t_y) + (\bar{t} - t_z)]$. Therefore, the test for significance consisted of a comparison between the means of $(\bar{t} - t_x)$ and $[(\bar{t} - t_y) + (\bar{t} - t_z)]$.

The mean of the paretic swim was 974.933 with a standard deviation of 540.292. The mean of the non-paretic swim was 558.787 with a standard deviation of 483.312. The null hypothesis stated that there would be no difference between $(\bar{t} - t_x)$ and $[(\bar{t} - t_y) + (\bar{t} - t_z)]$ or that the paretic and non-paretic swims are equal. Using N = 30 and 29 degrees of freedom a t of -5.211 was obtained which was

Table I

t Test Comparing the Means of the Fatigue Produced by the Paretic and Non- paretic Swims

Name	N	Sum X	Sum XX	Mean	Standard Deviation
Paretic Swim	30	29248.000	36980352.000	974.933	540.292
Non-Paretic Swim	30	16763.000	16140737.000	558.767	483.312

1) Degrees of Freedom = 29

2) T 4 = -5.211

3) P = 0.00002

4) The observed difference is significant at the .001 level.

significant at the 0.001 level. These results justify the rejection of the null hypothesis. The paretic and non-paretic swim did not have the same influence on the fatigue rate and a rat forced to swim under conditions of partial paralysis develops a fatigue which can not be explained in terms of either the muscle activity or the effect of the paralyzing drug.

A training effect could have produced an improved performance in t_y and in t_z such that the effect of the swim t_o and the drug taken separately would have caused t_y and t_z to be too large and $(\bar{t} - t_y) + (\bar{t} - t_z)$ too small. A difference between the paretic swim $(\bar{t} - t_x)$ and the non-paretic swim $(\bar{t} - t_y) + (\bar{t} - t_z)$ would then be, at least in part, an expression of a training effect. To investigate this possible source of error each rat was subjected to a marathon swim at the beginning and at the end of the experimental procedure. A t test was conducted on the means of the pre- and post-experimental marathon swims. The results are presented in Table II. The mean for the pre-experimental swim was 1483.833 seconds with a standard deviation of 673.259. The post-experimental swim mean was 1536.933 with a standard deviation of 603.831. With N = 30 and 29 degrees of freedom the t was 1.636 which was not statistically

Table II

t Test Comparing the Means of the Pre- and Post-experimental Marathon Swims

Name	N	Sum X	Sum XX	Mean	Standard Deviation
Pre-experimental Marathon Swim	30	44515.000	79197792.000	1483.833	673.259
Post-experimental Marathon Swim	30	46108.000	81438560.000	1536.933	603.831

1) Degrees of Freedom = 29

2) T 4 = 1.636

3) P - 0.11267

4) The observed difference is not statistically significant

significant. The results indicate that no training occurred between the pre- and post-experimental marathon swims.

Discussion

Analysis of the data compiled during the study suggests some conclusions which may be at variance with the usual concept of fatigue causality as previously reported. The time-honored assertion that fatigue is a peripheral dysfunction is rendered worthy of a more careful scrutiny. The results of the present study suggest that such a cursory assertion may represent an over-simplification of the fatigue phenomenon.

The hypothesis investigated by this study stated that exercise performed under conditions of partial paralysis would not induce a fatigue which varied quantitatively from the fatigue produced by exercise performed in the absence of partial paralysis or under normal conditions. Using student's t the hypothesis was unacceptable. Rats forced to swim under paretic conditions developed a fatigue which did differ quantitatively from the fatigue produced by a forced swim for a similar period of time but without the partial paralysis. The question is thus raised as to the mechanism by which the fatigue developed.

That fatigue can be produced as the result of attempted, but unrealized, movements is adequately supported by empirical observation of persons with various cerebellar lesions and certain other disorders of movement (4, p. 1286). Under such conditions it is conceivable that, in an attempt to force activity of an unresponding musculature, a greatly increased number of nerve impulses must be sent along lower motor neurons to realize an effective movement. As a result of the synaptic fatigue previously described (Chapter II) such neural effort may tax the ability of central synapses to maintain activity in lower motor neurons. The decrease in effective synaptic transmission would produce a concomitant decrease in muscle activity and the objective criterion of fatigue is realized.

The presence of weakness in cerebellar lesions noted above suggests that the subjective component of fatigue may involve the cerebellum. The weakness associated with Addison's disease and its therapeutic amelioration with glucocorticoids offers evidence that conditions, other than neural disorders, which disturb the motor response to nerve impulses may give rise to asthenia.

The physiological correlate of the subjective fatigue experience is not explained in the literature. The

cerebellar involvement suggests that the asthenia might be in part the function of a relationship between efferent motor activity and kinesthetic or proprioceptive feedback. The intricacies of these mechanisms are as yet poorly understood (17, p. 259). The role of the cerebellum in motor control is presented by Guyton (22, p. 817). When a movement pattern has been learned or exists as an innate capacity, a memory engram of the movement remains in the cerebral cortex and motor associated structures. This engram serves as a pattern for future performance of the same movement and, as the movement is made (for example one involving the fingers, hands, and arms),

> ...proprioceptive signals from the fingers, hands, and arms are compared with the engram, and if the two do not match each other, the difference, called the 'error', initiates additional motor signals that automatically activate appropriate muscles to bring the fingers, hands, and arms into the necessary attitudes for the performance of the task." (22, p. 817)

Nerve impulse transmission and muscle response in conformity with the engram could be the physiological correlate of the subjective experience "not fatigued." In any prolonged activity involving central synapses the possibility exists, and is supported by the present study, that central fatigue may occur. An increase in nerve impulses from the motor cortex would not be accompanied by appro-

priate and proportional changes in the proprioceptive feedback or input. The proprioceptive component of the engram can only be matched by a number of efferent impulses greater than was previously necessary if matching is possible at all. The efferent activity required as compared with that suggested by the engram are at variance. This variance may represent the physiological correlate of subjective asthenia.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Fatigue induction with minimal effort is characteristic of various disorders in which voluntary movements are rendered difficult due to aberrant neural activity or muscular response (See Chapters I and II). This study was undertaken in an attempt to determine if the fatigue observed under such conditions could be simulated through the artificial induction of paresis in otherwise normal animals. It is worth noting that in the various pathological states paralysis is not complete. Therefore the study was designed to approximate the partial paralysis or paresis observed in these disorders.

The methodology for the study involved first the use of a pilot study to determine the effects of the neuromuscular blocking agent and to become acquainted with the swimming characteristics of the rats employed in this sample. Subsequently the experiment was effected wherein the rats were forced to swim under normal and paretic conditions to evince any difference in the fatigue rate between these two swimming conditions. The effect of the paresis, induced with

succinylcholine, was separated from the effect of the drug, and the effect of the muscular effort incident to the swim, by the duplication of the drug effect and the swim effect in separate procedures. The effect of the drug and the effect of the swim were then subtracted from the fatigue produced by the drug, swim, and paresis operating in concert. The remaining fatigue was proportional to the paretic affect.

An analysis of the data collected during the course of the pilot study and experimental procedure revealed the following:

1. The onset of drug effect was slower, and the duration of effect longer, with intraperitoneal as opposed to intraveneous injection.

2. The response to the drug at uniform doses was variable among the individual rats tested.

3. A satisfactory paresis was induced when the succinylcholine was administered at a dosage of 1.30 mg per kilogram of body weight.

4. Dyspnea and cyanosis were more readily controlled when the drug dosage was administered in five equal increments.

5. Under normal conditions, in 77 per cent of the animals tested, the rats swam for a period of less than

thirty minutes when weighted after the manner of Hardin (49, p. 370).

6. No training effect or improvement in swimming performance was noted over the five swimming procedures to which the rats were subjected.

7. The fatigue induced by swimming under paretic conditions differed quantitatively from that induced under normal swimming conditions and the difference was significant at the .001 level of confidence.

8. A period of seventy-two hours between procedures allowed sufficient recovery from the effects of the drug and swimming to produce a marathon swim equivalent to the initial swim.

Conclusions:

On the basis of the above findings the following conclusions can be made:

1. Onset of succinylcholine action will be slower and of longer duration when the drug is injected intraperitoneally as opposed to intraveniously.

2. The individual response to the drug is variable as has been previously reported by Goodman (29, p. 615).

3. Paresis can be induced with a specified drug

dosage as it relates to body weight.

4. Dyspnea and cyanosis may be controlled when the drug dosage is administered in five equal increments.

5. Rats weighted at three per cent of body weight may be expected to swim for less than thirty minutes as predicted by Hardin (23, p. 370).

6. Over a period of five swimming sessions, rats will not increase their swimming time as a result of training.

7. The fatigue induced by swimming under paretic conditions is greater quantitatively than the fatigue developed under normal swimming conditions. The null hypothesis is rejected.

8. A seventy-two hour recovery period between procedures allows complete dissipation of any drug or swimming influence on subsequent swimming performance.

Recommendations:

On the basis of the study the following recommendations are offered:

1. A study of fatigue under conditions of paresis in humans would be appropriate to determine whether similar mechanisms might be operative.

2. Research needs to be conducted to evaluate the influence of amphetamines on the fatigue induced by paresis.

3. A study should be conducted to determine the effect of paretic fatigue on isolated muscle activity such as was utilized in the studies of Merton and Stevens previously cited.

4. Development of fatigue under conditions of partial paralysis should be investigated in other forms of exercise such as running and weight lifting.

5. It would be desirable to conduct similar studies employing the use of surface or inserted electrodes to compare muscle and nerve activity under conditions of paresis.

6. A study comparing fatigue rate with efferent motor and afferent proprioceptive activity would be appropriate.

7. Research investigating the influence of a conditioned stimulus administered during paralysis on subsequent fatigue rate would disclose more information on the "engram" hypothesis.

8. The influence of other drugs (caffeine, alcohol, etc.) on paretic fatigue needs to be studied to ascertain the possible influence of such drugs on the asthenia of this origin.

9. A study of motor cortical activity as compared with muscle response is needed to ascertain any relationship between this ratio and fatigue development.

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APPENDIX

FATIGUE TIMES (IN SECONDS) FOR THE EXPERIMENTAL PROCEDURES

Rat Id. Rat, Cage	Swim to Fatigue = t_1	Swim t_o + Drug a = t_x	Swim $t_o = t_y$	Drug $a_1 = t_z$	Swim to Fatigue = t_2	$\bar{t}$	$\bar{t} - t_y = a$	$\bar{t} - t_z = c$	$\bar{t} - t_x = b$	a + c	b -(a + c)	t_o
1, 1 ♂	849	Died	---	---	---	---	---	---	---	---	---	---
1, 2 ♂	797	260	545	688	860	828	283	140	568	423	145	135
2, 2 ♂	Non-swimmer	---	---	---	---	---	---	---	---	---	---	---
1, 3 ♂	1,607	838	1,425	1,510	1,718	1,662	237	152	824	389	435	255
2, 3 ♂	2,120	950	1,850	1,625	1,995	2,057	207	432	1,107	639	468	230
3, 3 ♂	2,885	1,095	2,285	1,590	2,558	2,721	436	1,131	1,626	1,567	59	265
1, 4 ♂	1,235	645	735	1,128	1,145	1,190	455	62	545	517	28	225
2, 8 ♂	1,655	180	1,375	715	1,693	1,674	299	959	1,494	1,258	236	88
1, 8 ♂	490	288	440	410	385	437	---	27	149	27	122	215
2, 14 ♀	2,830	965	2,734	2,524	2,692	2,761	27	237	1,796	264	1,532	110
3, 14 ♀	2,595	885	2,076	1,500	2,403	2,499	423	999	1,614	1,422	192	170
1, 14 ♀	3,285	Died	---	---	---	---	---	---	---	---	---	---
2, 15 ♀	1,210	663	840	1,141	1,370	1,290	450	149	627	599	28	295
3, 15 ♀	1,027	935	1,380	1,200	1,700	1,363	---	163	428	163	265	135
1, 15 ♀	780	387	1,440	1,248	875	827	---	---	440	0	440	135
2, 17 ♀	1,972	690	2,035	1,940	1,862	1,917	---	---	1,227	0	1,227	130
3, 17 ♀	877	155	730	1,115	938	907	204	---	752	204	548	130
1, 17 ♀	1,497	435	1,330	860	1,575	1,536	206	676	1,101	882	219	315
2, 1 ♂	990	375	550	903	955	972	422	69	597	491	106	140
3, 1 ♂	1,358	503	1,150	965	1,325	1,341	191	376	838	567	271	210
3, 2 ♂	1,125	615	984	885	1,166	1,145	161	260	530	420	110	125
2, 4 ♂	1,385	518	882	1,155	1,465	1,425	543	270	907	810	97	95
3, 4 ♂	2,325	765	2,360	1,075	2,253	2,289	---	1,214	1,524	1,214	310	245
2, 12 ♂	1,637	870	1,695	1,595	1,710	1,673	---	78	803	78	725	205
2, 13 ♀	832	385	768	735	928	880	112	145	495	257	238	110
2, 10 ♂	2,735	355	2,525	1,470	2,838	2,786	373	1,316	2,431	1,689	742	140
3, 10 ♀	815	145	501	945	953	884	383	---	739	383	356	82
3, 14 ♀	1,220	525	1,358	680	1,295	1,257	---	577	732	577	155	155
2, 14 ♂	1,440	375	1,555	1,660	1,700	1,570	15	---	1,927	15	1,912	110
1, 14 ♀	1,380	160	1,040	943	1,483	1,431	391	488	1,271	879	392	130
1, 15 ♂	1,985	763	1,835	1,350	2,050	2,017	182	667	1,254	849	405	230
2, 5 ♀	645	325	590	950	895	770	180	---	445	180	265	200
1, 5 ♀	1,066	737	1,290	1,200	1,323	1,194	---	---	457	0	457	175