A Comparison of Normal, Vitamin E Deficient and Denervated Skeletal Muscle in Experimental Animals By Means of Electrodiagnostic, Electromyographic and Pharmacologic Criteria

John Joseph Fudema
Loyola University Chicago

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A COMPARISON OF NORMAL, VITAMIN E DEFICIENT AND DENERVATED
SKELETAL MUSCLE IN EXPERIMENTAL ANIMALS BY MEANS
OF ELECTRODIAGNOSTIC, ELECTROMYOGRAPHIC
AND PHARMACOLOGIC CRITERIA.

by

John Joseph Pudena

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy

June
1958
APPROVAL SHEET

The dissertation submitted by John J. Fudema has been read and approved by five members of the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

29 May 1958

[Signature of Adviser]
John Joseph Fudema was born in Chicago, Illinois, February 25, 1923.

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He is author and co-author of the following publications:


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CHAPTER I

INTRODUCTION

A. Statement of the Problem.

The histological changes in the skeletal muscles of experimental animals on a vitamin E deficient diet have been extensively studied since their discovery by Goettch (1930) and Pappenheimer (1930). Subsequently, it was observed (Adams et al.) that a similarity in the pathologic picture obtains between skeletal muscle of the vitamin E deficient animals and that of progressive muscular dystrophy in humans, even though vitamin E (alpha-tocopherol) administration is without benefit in relieving human dystrophy symptoms.

In recent years the concern of investigators has been the biochemical aspects of the experimental and human dystrophy problem (Milsam, 1955; Schapira, et al., 1955; Shull et al., 1957). A review of the literature reveals that little attention has been given electrophysiologic responses in these myopathies.

Since there is a lack of definitive information concerning the electrophysiology of vitamin E deficient skeletal muscles of experimental animals, an investigation was undertaken to study the electrophysiologic changes in E deficient muscle using electrodiagnosis and electromyography criteria.

In preliminary studies, a finding was made not previously reported
in the literature: spontaneously occurring fibrillation potentials were observed electromyographically in the skeletal muscles of vitamin E deficient rabbits. This drew attention to the similarity which obtains between these potentials and the fibrillation potentials seen in denervated skeletal muscle.

In view of this finding, it was decided that electrophysiologic data should also be collected from a group of chronically denervated animals which would allow a comparison to be made with data from muscles of E deficient animals.

It was also deemed desirable to compare the fibrillation potentials of E deficiency and of denervation pharmacologically. Several neuro-muscular drugs (prostigmine, d-tubocurarine, decamethonium, and succinylcholine) have been reported to have an effect on fibrillation potentials of denervated muscle (see "Related literature.") As the fibrillation potentials occurring in vitamin E deficient skeletal muscle have not been reported in the literature, no comparable study could have been made on the effect of these neuro-muscular agents on these potentials. A study was undertaken to compare the effects of these neuro-muscular drugs on the fibrillation potentials of E deficient and of denervated muscle.

Finally, an acute weight loss was observed to occur in the experimental animals in the late stages of the E deficiency syndrome concomittant with the changes in electrodiagnosis data. To determine whether or not inanition alone may affect the electrophysiologic findings, electrodiagnosis and electromyography data were collected from a group of normal rabbits acutely fasted on a low calorie diet but one which contains vitamin E.
Summarizing the investigation to be undertaken:

Electrodiagnosis and electromyography data will be collected from the anterior tibial muscles of vitamin E deficient rabbits and contrasted against a background of comparable data obtained from: (1) Control animals on the same diet with vitamin E, (2) denervated animals maintained on the control diet, and (3) acutely fasted animals on a low caloric diet but one which contains the vitamins and minerals found in the control diet.

Pharmacologically, the fibrillation potentials seen in the E deficient and in denervated muscles will be compared using several neuro-muscular drugs.

B. Related literature.

1. Vitamin E effects.

Our knowledge of vitamin E dates from 1922, when Evans and Bishop showed that wheat germ oil contains a factor (substance "x", later called vitamin E) necessary for successful completion of pregnancy in female rats, and for the preservation of spermatogenesis in the male rat. Advances in the chemistry of these compounds have continued to the present, at which time at least seven distinct tocopherols have been identified. In addition, many synthetic compounds are reported to have vitamin E activity. These include the phosphate and acetate esters and the 5, 7-dimethyl-8-ethyl homolog of tocopherol (West and Mason, 1955).

During the period 1923 to 1929, interest in vitamin E was primarily focused on (1) the effects of vitamin E upon reproduction, (2) its occurrence
in various plants and in animal tissues, and (3) its chemical constitution, identification and synthesis. In 1926, Evans and Burr described a paralysis which developed in suckling rats of vitamin E deficient mothers. The production of lesions in skeletal muscle by a vitamin E deficient diet was first recognized by Coettsch and Pappenheimer. These workers were able to produce lesions in the skeletal musculature of guinea pigs and rabbits. They found that the guinea pigs develop extreme degeneration of the skeletal musculature of the trunk and extremities. The primary alteration in the histological picture consists of a waxy or hyaline necrosis of the fibers, followed by a great proliferation of the nuclei within the intact sarcolemmas. There is also active regeneration of muscle cells in the later stages with the disappearance of the degenerating fibers accompanied by a variable amount of interstitial fibrosis and lipomatosis. The affected muscles were characterized grossly by a striking pallor, the muscle tone and elasticity were lost, the muscle was "without irritability", and the muscle bulk was much reduced in comparison with that of control littermates. It was concluded that the experimental diet brings about a generalized dystrophy of the entire voluntary muscle system with no significant lesions found in any other tissue or organ. No scurbutic lesions were found. Insiniten was excluded as a possible factor, since in their 1931 paper, Coettsch and Pappenheimer report having subjected animals to starvation with no effect on the skeletal muscle histology.

That the muscle lesions were a manifestation of vitamin E deficiency was not at first clearly appreciated because wheat germ oil as a source of
vitamin E, in the doses used (which were insufficient), did not prevent the disease. Later, with pure tocopherol available, it was amply shown by Mackenzie and McCollum, 1940, that this experimental muscular dystrophy could be ascribed to vitamin E lack. These authors found that nutritional muscular dystrophy in the rabbit resulting from a deficiency of a "fat-soluble factor" is cured by administration of alpha-tocopherol.

Rogers, Pappenheimer and Goettach, 1931, report that the nerve endings in muscular dystrophy in guinea pigs are preserved even though the muscle fibers are profoundly altered. Pappenheimer, 1939, writes that "terminal neurites and end-plates were well preserved in the midst of degenerated muscle fibers" in E deficient rat skeletal muscle. This finding was also confirmed for rabbit skeletal muscle by Gatz and Houchin (1951) and Anderson and Rickard (1957).

Since the initial discovery of nutritional muscular dystrophy in guinea pigs and rabbits by Goettach and Pappenheimer in 1930, this same condition has been produced in a variety of animals including: monkeys (Mason and Telford, 1947); dogs (Anderson et al., 1939); sheep and goats (Nadsen et al., 1935); ducks (Goettach and Pappenheimer, 1934); hamsters (Houchin, 1942); rats (Ringeled, 1935); and mice (Pappenheimer, 1942).

Not all species show specific skeletal muscle damage. For example: rabbits (Gatz and Houchin, 1951) and cattle (Gullickson and Calverly, 1946) showed changes in cardiac muscle; "nutritional encephalomalacia" has been described as occurring in young chicks by Pappenheimer and Goettach in 1931; Dam and his associates (1939, 1942) described "exudative diathesis" in chicks; and in turkeys, vitamin E deficiency effects are restricted to the smooth muscle of
the gizzard (Jungherr and Pappenheimer, 1937).

2. Electrodiagnosis.

Electrodiagnostic examination of normal and denervated muscle in humans and experimental animals is well documented. The procedures commonly used in routine electrodiagnosis examinations, and the ones selected for the present study, include: rheobase, strength-duration, chronaxie, repetitive stimulation, galvanic tetanus ratio, and response to faradic stimulation.

The only report in the literature describing the application of any of these procedures to a study of E deficient skeletal muscle appears to be that of Victor (1934). This investigator studied "strength-duration curves" in E deficient rabbits and ducks. What this author actually reported on was only two points on the strength-duration curve, namely, rheobase and chronaxie. He concluded that rheobase and chronaxie of dystrophic muscle of rabbits and ducks is greater than normal and that "a statistical analysis of the irritability changes is futile because of the great variation in the degree and type of change in the muscles".

The value of rheobase as a diagnostic guide appears to be questionable. It has been reported that rheobase decreases in denervation, with a sudden rise heralding recovery in peripheral nerve injury (Laroquette, 1920; Pollock et al., 1945). Ritchie, 1954, did not find that rheobase was low in denervated muscle nor did it tend to rise in recovery. Wynn-Parry, 1953, reported variable results. The technical factors (electrode size and position) and local conditions of the tissue (skin temperature and preparation, blood
supply, edema, and exercise) as they influence the rheobase were extensively investigated by Harris, 1952.

A twitch contraction occurs when a muscle is stimulated by the closing or opening of a circuit conveying direct current of liminal strength and long duration (rheobase). If a sufficiently strong current is applied to a muscle a prolonged excitation occurs producing persistent contraction or "tetanus". The earlier electrophysiologists (Bezold, Hering, Remak and others) termed this tetanus state "galvanotonus". Erb (1868), Mendelsohn (1909) and Roberts (1916) in studies made upon denervated muscle found that "galvanotonus" occurred when the muscle was stimulated with a threshold current (rheobase) or one slightly higher than threshold value. Pollock et al (1945b) proposed that since the tetanus results from galvanic stimulation it be called "galvanic tetanus". And as the absolute value of the threshold stimulus for tetanus may be quite high when the rheobase is high because of changes in the tissue (edema, inflammation, etc.) it is more important to estimate the ratio between the threshold stimulus for tetanus and rheobase. This ratio has been called "polarization coefficient" by Zamistowski (1915). Since it is the ratio between the threshold amperage of galvanic current to produce tetanus and rheobase at the same current duration, Pollock proposed that it be called the "galvanic tetanus ratio".

Pollock et al (1945) reported on the galvanic tetanus ratio changes of the gastrocnemius muscle of cats in which a nerve section with immediate suture of the sciatic nerve had been performed. These authors summarized galvanic tetanus ratio and the status of innervation as follows: In normal
animals the galvanic tetanus ratio range may be 3.5 to 6.0. Denervated muscle may have a range of from 10.0 to 1.0. The complete denervation state of muscle is present when the galvanic tetanus ratio is 1.0 to 1.5. In reinnervation after primary nerve suture, the galvanic tetanus ratio value rises from 2.0 to 20.0 and then returns to normal. In view of the shifts in these curves, Pollock cautions that "in the interpretation of the data from examination for galvanic tetanus, the time which has elapsed from the date of injury or operation is of considerable importance".

In strength-duration studies, Lucas (1906, 1907-08) found that by using nonpolarizable electrodes and stimulating sartorius muscles of toads and frogs with galvanic current, "complex" strength-duration curves could be obtained. The components of this type of curve, which consisted of two and at times three distinct segments, Lucas identified as the response of (1) muscle fibers to currents of long durations, (2) nerve fibers to currents of short duration, and (3) "myo-neural junction" response to currents of very short duration. Using the same type of electrodes, Rauhut (1932) confirmed the presence of at least two excitabilities with different time relationships -- that due to the muscle fiber and that due to the nerve fiber.

Adrian, 1916, in clinical and experimental studies on strength-duration curves of normal and denervated muscle, found that normal muscle possesses a "rapid" and continuous curve; and that denervated muscle in the process of recovery following nerve suture yields a "discontinuous" curve with two components. Adrian pointed out that with very strong currents the discontinuous curve has the short time characteristics of healthy muscle, but with
weaker strengths a slower curve appears, and this has the long time constants of denervation. He proposed that in the production of these double curves there are two distinct mechanisms upon which the current may take effect. He believed that these two mechanisms were to be identified with the muscle fibers and the nerve fiber or nerve ending.

Pollock and his co-workers (1945, a,b) recognized the practical significance of the findings of the denervation studies by Adrian. They point out that the presence of the discontinuity in the strength-duration curve provides a method for discovering the persistence of functional nerve fibers in nerve or muscle injury. In experimental studies they found that during the period of nerve degeneration following nerve section, before the muscle is "completely" denervated, discontinuities may appear. Further, the discontinuity disappears and the strength-duration curve becomes continuous when the muscle is "completely" denervated.

Lapicque (1906) recognized that the relation between various strength-duration curves taken at different times and with different subjects differed so greatly as to make comparison difficult. To facilitate studies of muscle in normal and abnormal states he proposed the selection of a function of the rheobase as a numerical expression of excitability. He termed this function "chronaxie" and defined it as the minimum time required to excite tissue for a stimulus of twice the strength of the rheobase.

Lapicque (1906) found that the chronaxie of different normal muscles in the human varied from 0.2 to 0.5 milliseconds. The chronaxie values
determined by subsequent experimental investigations on normal muscles (Lucas, 1907-08; Adrian, 1916; Davis, 1922-23) and denervated muscles (Watts, 1924-25; Pollock et al., 1945a) may be summarized as follows: In the normal (muscle), the chronaxie is very short (1.0 milliseconds or less); in the completely denervated state, the chronaxie is long reaching figures as high as 100 milliseconds.

Repetitive stimulation is a means of producing a tetanic contraction of muscle by use of a series of interrupted stimuli. Typical findings in electro-diagnosis with repetitive stimulation have been reported by Pollock et al. (1947) and in a monograph published by the Meditron Corporation (1952). In normal muscle, repetitive stimuli of the three frequencies used (500, 166, and 77 cycles per second) require about the same amount of current to produce tetanic contraction. In denervated muscle, progressively greater amounts of current are required as the frequency of the stimulus decreases.

Faradic current consists of a rapid series of very short bi-phasic impulses (100 to 300 cycles per second) and is usually obtained from an induction coil. It has limited application in electrodiagnosis because it is impossible to measure accurately. Summarizing the work of earlier investigators of the effects of faradic stimulation on muscle (Erb, 1868; Roberts, 1916; Adrian, 1916; and Mayfield et al., 1945), stimulation of muscle by faradic current produces contraction of muscle only when the nerve can conduct impulses. Faradic stimulation will always induce contraction in normal muscle or within the first fifteen days after denervation.
Pollock et al. (195b) disagreed with the findings of these earlier workers. They report that in the experimental animal (cat) excitation by faradic stimulation persists throughout the period of denervation and muscle reinervation, both when the stimulus is applied percutaneously and on the exposed muscle. In man, exposed muscle continues to contract when stimulated by faradic current and if the necessary strength of current were tolerable to the subject it probably would produce, and has been found to produce, contractions due to percutaneous stimulation. Second, many cases show motor recovery long before percutaneous faradic stimulation produces contraction. Third, in spontaneously recovering lesions, destined to recover in a period of time less than that which would be required were the part of the nerve distal to the injury completely degenerated, failure to respond to faradic stimulus is common.

3. Electromyography.

To date, no reports have appeared in the literature relating the application of electromyographic techniques to a study of skeletal muscle in experimental vitamin E deficiency.

Normal and denervated muscle have been extensively studied electromyographically. The findings of some of these studies may be briefly summarized as follows:

Electromyographic recording of a normal skeletal muscle at rest reveals an undisturbed iso-electric base-line usually referred to as "electrical silence". Electrical silence implies complete neuromuscular relaxation and is a significant "normal" finding. When denervated, as with
damage to or section of the motor nerve, the muscle fiber undergoes various structural and functional changes, and in the space of a few days, fine spontaneous movements of the muscle fiber appear. These movements were first observed grossly by Schiff (1951) who termed them "fibrillations". Concomitantly, the electrical silence of resting muscle seen electromyographically is replaced by repetitive potentials of small amplitude (50 to 150 microvolts) and very short duration (1 to 2 milliseconds). It has been concluded by Denny-Brown and Pannapacker (1936) as well as many subsequent investigators that these spontaneously occurring potentials represent activity in single fibers, and is found to be present in denervated muscle only.

Normal neuromuscular function is represented electromyographically by the production of motor unit potentials. The motor unit is defined as the motor nerve cell, its axon, end-plates and all the muscle fibers it innervates. The axon of a motor neuron divides many times forming terminal neurites which supply many muscle fibers. Thus, an impulse propagated along a motor neuron may cause all the muscle fibers innervated by that neuron to contract simultaneously as a "unit" producing a smooth and summated motor unit action potential. Consequently, all activity of normal skeletal muscle is based upon the integrity and organization of the motor unit. Immediately following motor nerve section no motor units may be activated voluntarily by the subject. In specific myopathies alteration in the wave form, sound, and parameters may yield information as to the particular type of pathology which is present (Licht, 1956).
4. **Experimental pharmacology with neuromuscular drugs.**

One of the significant findings in this investigation was the presence of spontaneously occurring fibrillation potentials in the skeletal muscles of the vitamin E deficient animals. Electromyographic findings were that no difference could be discerned between the electrical parameters of the fibrillation potentials of E deficient muscle and the fibrillation potentials obtained from skeletal muscle of denervated rabbits. Several drugs, which are said to act primarily at the myoneural junction, have been studied in terms of their effect on the fibrillatory activity of denervated muscle. These include d-tubocurarine, decamethonium, succinylcholine, and prostigmine. As the presence of fibrillation potentials in E deficient muscle represents an original finding, no data is available in literature of a comparable study with these neuromuscular pharmacologic agents on this fibrillation activity. As part of this investigation, a study was made of the effects of these drugs on the fibrillation potentials of vitamin E deficient and of denervated skeletal muscles of rabbit using electromyographic techniques.

Some of the background literature relating to drug effects on denervation fibrillation may be summarized as follows:

The first reported work dealing with the effect of curare on fibrillation of denervation appears to be that of Langley and Kato, 1914-15. By visual observation of exposed denervated gastrocnemii muscles of rabbits, they found that fibrillation was not stopped by curare. Ten days after denervation in a rabbit weighing 1.2 kilograms, curare was given in successive doses up to
7.0 cc of a 1 per cent solution, and the fibrillation was "still lively". The
curare solution was reported as being "made fresh from a purified curari ex-
tact, and was very active; 1.0 cc of it paralyzed the motor nerves in another
rabbit weighing 1.25 kilograms.

Using electromyographic techniques on denervated muscles of experi-
mental animals, Solandt and Magladery (1940), Eccles (1941) and Reid (1942),
found that curare is without effect on fibrillation when administered in
paralyzing doses. Quoting Reid, "fibrillation is unaffected by doses of curare
which in the same muscle reduce the response of denervated muscle to acetyl-
choline."

In 1945, McIntyre working with denervated dog muscle reported that
d-tubocurarine in doses not causing complete muscle block does not effect
fibrillation. Intra-arterial injection of a very large dose of d-tubocurarine
is capable of producing prolonged muscle contractures and during this contrac-
ture the electrical fibrillation of denervation is markedly reduced and some-
times completely depressed.

Jarcho et al., 1950, observed the effect of d-tubocurarine on the
 electromyographic picture of denervated gracilis muscle in the rat. They found
that minimal doses had no effect, while larger doses produced a decrease in
voltage and frequency of fibrillatory response but never total obliteration.
Following up on this work, they reported in a 1951 paper that in denervated
gracilis muscle of the rat, less than normal blocking doses of d-tubocurarine
caused an increase in fibrillatory activity; normal blocking doses brought
about a decrease in fibrillation; and in doses several times the blocking dose, fibrillation ceased.

Jarcho et al., 1950, reported that in denervated rat muscle, intravenous injection of small amounts of decamethonium (one-twentieth of the blocking dose) caused an almost immediate (within 5 to 10 seconds) outburst of electrical "fibrillary activity" rapidly followed by complete silence. After this depression, there were on occasion, variable periods of increased activity, and then a return to control levels of fibrillation. The initial outburst of increased "fibrillary activity" was ascribed to the depolarization effect of this drug; the cessation of activity to the persistent depolarization. This effect was confirmed by Naisse, 1951, for denervated cat muscle. She found that close intra-arterial injection of a small dose of decamethonium was accompanied by an outburst of electrical activity greatly in excess of any previous spontaneous activity. As the dose was increased the immediate outburst of potentials stopped abruptly and no activity was present.

Riker et al. (1957) studied the effect of several "depolarizing agents on an isolated fibrillating denervated muscle fiber in the intact cat. They found that the frequency of the "single" repetitively firing fibrillation potential is "sharply but briefly increased by decamethonium depending on the dose. They point out that "if the dose is sufficiently large, a maximal discharge abruptly ceases and quiescence prevails".

No studies have been reported on the effect of succinylcholine on fibrillation potentials of denervation.
In 1937, Rosenbluth and Lucé reported an electromyographic study of the effect of prostigmine on denervated cat muscle. They reported an increase in number and magnitude of fibrillation potentials. In 1946, Riker and Wescott reported similar findings.
CHAPTER II

MATERIALS AND METHODS

A. Experimental animal.

Littermate New Zealand giant rabbits (males), weighing about 1000 grams, were the subjects of the vitamin E deficiency experiment. Rabbits were chosen because they respond well to the deficiency diet and they are the animal of choice of many previous investigators, hence background material on vitamin E deficiency effects is available for comparison.

B. Diet material.

1. Experimental diet.

The following low vitamin E diet was prepared to produce experimental vitamin E deficiency: Non-nutritive fiber 20%, casein (vitamin free) 13.5%, Sucrose (as granulated sugar) 42%, lard 5%, yeast (dried brewers') 13.5%, cod liver oil 3% and salt mixture 3%.

The diet materials bear the following specifications:

Non-nutritive fiber. Consists of a vegetable cellulose powder for use as a non-nutritive filler in diet materials and has no available food value. Prepared by General Biochemicals, Inc.
Casein. Described as a vitamin test casein which has undergone several extractions with hot alcohol to remove fat and water soluble vitamins. Prepared by General Biochemicals.

Sucrose. Commercial extra fine granulated sugar.

Lard. With not more than: .0077% butylated hydroxyanisole, .0077% butylated hydroxytoluene, .0046% propyl gallate, .0100% citric acid (as preservatives: Propylene glycol and mixed glycerides).

Yeast. Containing: Vitamin B¹ (Thiamin) 200 International units per gram; Vitamin C, 70 gamma Riboflavin per gram; as well as Niacin and other factors of the B complex natural to yeast. Prepared by the Chicago Dietetic Supply House, Incorporated.


Salt mixture. Composition:

<table>
<thead>
<tr>
<th></th>
<th>Grams</th>
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</thead>
<tbody>
<tr>
<td>Calcium carbonate</td>
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<tr>
<td>Magnesium carbonate</td>
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</tr>
<tr>
<td>Magnesium sulfate</td>
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<td>6.900</td>
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<tr>
<td>Potassium chloride</td>
<td>11.200</td>
</tr>
<tr>
<td>Potassium phosphate (mono-basic)</td>
<td>21.200</td>
</tr>
<tr>
<td>Ferric phosphate</td>
<td>2.050</td>
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<tr>
<td>Potassium iodide</td>
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</tr>
<tr>
<td>Manganese sulfate</td>
<td>0.035</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>0.100</td>
</tr>
<tr>
<td>Aluminum potassium sulfate</td>
<td>0.017</td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>0.090</td>
</tr>
</tbody>
</table>

Prepared according to the recommendation of Hubbel, Mendel and Wakeman, 1937, by General Biochemicals, Incorporated.
The dry ingredients were thoroughly mixed in a large tub; the cod liver oil and lard (melted but not hot) were then mixed in. The diet material was stored under refrigeration.

The animals were fed daily and allowed to partake of the diet ad libitum. Feed and water bowls were washed clean daily.

2. Control diet.

The control animals received the same ration as the experimental animals, but supplemented with alpha-tocopherol (sphynol acetate) 50 milligrams per kilogram of diet.

C. Design of the experiment.

In order to obtain nearly homogenous data which would lend themselves to statistical analysis, it would have been desirable to examine all animals at the same time on a given day. Since, however, this could not be done because of limitations in equipment and personnel, the examinations were scheduled in groups, closely spaced in time. To minimize the probability of a systematic factor entering into the routine of examining the animals, they were assigned to placed in each group of examinations by use of random number tables.

It was expected that the average of the data obtained from each of these groups of examinations would be representative of the condition of the animals at the middle of this time interval. This would be regarded as true until the variance of some of the measurements increased significantly forcing
us to admit the presence of non-homogeneous data. As long as the data appeared
to be homogeneous, they could be averaged in a meaningful way and the limits
to their distribution could be stated. When the variance increased, the data
for animals could be separated and we would look at the behavior of individ-
ual animals instead of looking at the average of a group.

A preliminary experiment had suggested that the time of most rapid
change in electrodiagnostic characteristics would be from twenty-nine to
forty-six days after the entry into the vitamin E deficient diet. From this
information a schedule of examinations was prepared. As the experiment
progressed, it was found that the particular population of rabbits being used
for this program entered into the period of rapid change earlier than had
been expected and hence a slight revision of the schedule of terminal
examinations was necessary. (See Table I for the schedule actually followed.)

A schedule for the examination of the control animals was established
(as shown in Table I) so that their data would be obtained in groups comparable
to those of the experimental animals.

Electrodiagnosis criteria listed under "Electrodiagnosis Procedure"
are to be studied. These numerical findings would be expressed in units of
time and current in all cases except for the results of faradic stimulation.
The units of time measured the duration of flow of the stimulating current.
The time values were to be the independent variables in all cases, that is,
pre-determined durations of stimuli were to be used. The dependent variable
which would be determined by the procedure would be, in all cases, the
magnitude of the current in milliamperes required to produce the specified
### TABLE I

**SCHEDULE OF EXAMINATION DAYS FOR VITAMIN E DEFICIENT RABBITS AND THEIR CONTROL RABBITS**

<table>
<thead>
<tr>
<th>Examination Group</th>
<th>Experimental Diet Rabbits</th>
<th>Control Diet Rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series I</td>
<td>2, 3, 4</td>
<td>2, 3, 4</td>
</tr>
<tr>
<td>Series II</td>
<td>8, 9, 10, 11</td>
<td>8, 9, 10, 11</td>
</tr>
<tr>
<td>Series III</td>
<td>17, 18, 19, 20</td>
<td>17, 18, 19</td>
</tr>
<tr>
<td>Series IV</td>
<td>21, 22, 23</td>
<td>27, 28, 29</td>
</tr>
<tr>
<td>Series V</td>
<td>24, 25 <em>at seg.</em> ad mortem</td>
<td>43, 44, 45</td>
</tr>
</tbody>
</table>

*FIGURES IN THE TABLE INDICATE THE NUMBER OF THE DAY SINCE THE RABBITS BEGAN THEIR RESPECTIVE DIETS.*

contraction. It would have been convenient if the current values for all rabbits receiving a given stimulus could be averaged. This, however, would not be meaningful since the threshold for a muscle at a given duration of stimulus in a normal rabbit is not a constant from one examination period to the next, although repeated thresholds are usually similar. To make a comparison from one rabbit to another, and also from one day to the next, it was decided to use a ratio of the threshold at the selected duration to the threshold at so-called long or "infinite" duration (taken here to be 300 milliseconds). This last value, threshold at long or "infinite" duration is
spoken of as the rheobase. The quotient, then, of the magnitude of current (in milliamperes) required to produce threshold response at a selected duration of stimulus divided by the magnitude of current (in milliamperes) required to produce rheobase response we called the "threshold ratio". It may be represented as follows:

\[
\text{Threshold Ratio} = \frac{\text{threshold current in milliamperes at specified duration}}{\text{threshold current in milliamperes at "infinite" duration (300 milliseconds)}}
\]

1. Influence of pilot studies.

Pilot studies on eighteen vitamin E deficient rabbits indicated three areas for further documentation: (1) A change in the character of voluntary motor unit potentials and the presence of spontaneous "fibrillation-like" potentials not under voluntary control but resembling those seen in lower motor neuron involvement (denervation-fibrillation potentials); (2) a change in the strength-duration curves including a lengthening of the chronaxie; and (3) a marked weight loss (average of 21.6% of the total body weight in five days). Since changes in electrodiagnosis data occur at about the same time as marked weight loss begins, the possibility exists that the electrodiagnostic changes may be due to inanition alone. It was decided to collect data on a group of fasting animals not on an E deficient program but with a comparable weight loss (about 20 per cent) during a comparable period of time (4 to 6 days).

The presence of the spontaneous "fibrillation-like" potentials with their resemblance to denervation fibrillation potentials pointed to the
desirability of comparing the two types of potentials electromyographically and pharmacologically. Therefore, it was decided to compare the findings in vitamin E deficient rabbits with a group of rabbits in which the common peroneal nerve was sectioned, resulting in chronic denervation of the anterior tibial muscle.

2. Choice of experimental conditions.

a. Chronic denervation study.

(1) Experiment design.

Seven normal male rabbits are to be the experimental subjects. Only the tibialis anticus on one side was to be denervated by section of the common peroneal nerve on that side. The contralateral muscles are to be used for control or reference. Both electrodiagnostic and electromyographic observations are to be made on the following post-operative days: First, fourth, eight, eighteenth and thirtieth.

The electrodiagnostic data are to be processed by expressing them in terms of "threshold-ratio" units as described in the "Design of the Experiment" for the vitamin E deficiency data. The electromyographic data are to be recorded with photographs and magnetic tape.

(2) Denervation technique.

The right tibialis anticus muscle was denervated in all cases. The common peroneal nerve was exposed surgically by sterile technique in the anesthetized animal (pentobarbital, 30 mg/kg for tranquilization; ethyl ether to achieve muscle relaxation). The common peroneal nerve was tied off two
centimeters above the tibialis nerve and divided. The central stump was reflected back onto itself and tied down in this position to delay and minimize reinnervation. 100,000 units of Penicillin G (intramuscular) was injected post-operatively as a prophylaxis against infection. The animals were maintained on the control diet.

b. Fasting study.

(1) Experiment design.

Seven normal male rabbits were the subject of this fasting study. Complete electrical examinations were to be made on the first day to provide control or reference data. Then a second set of examinations was made on each animal on the day that he was found to have lost approximately 20 per cent of his initial total body weight.

(2) Procedure.

Animals were weighed initially. Desired insanition weight was calculated for each animal. Animals were weighed daily while on the fasting diet to determine the end-point of the weight study (loss of 20 per cent of initial weight to match the average weight loss of the vitamin E deficient animals).

A calorically low diet, but one which contained all of the vitamins and salts of the control diet, was prepared. Its composition was as follows: Yeast 50.2%, cod liver oil 11.3%, ephynal acetate 1.0%, sucrose (sugar) 25.0%, and salt 12.5%. Twenty grams of this diet were fed to each of the isolated animals daily. The animals were allowed water ad libitum.
D. Electrodiagnosis.

1. General considerations.

Electrodiagnosis may be defined as the procedure in which one sends through a muscle or its motor nerve an electrical current of suitable intensity and selected wave form in order to obtain diagnostic information concerning neuromuscular disorders. While there are many types of data which may be obtained in a routine electrodiagnosis examination, the procedures selected for this present study include: Rheobase, Strength-Duration Curve, Chronaxie, Galvanic Tetanus Ratio (Cathodal), Repetitive Stimulation, and Response to Faradic Stimulation.

Rheobase is defined as the minimum amount of galvanic current lasting "infinite time" (300 milliseconds or more) which causes a reproducible threshold contraction.

The Strength-Duration Curve is a graph obtained by plotting the current required to produce the aforementioned threshold contraction observed in the determination of Rheobase, against a series of decreasing stimulus durations.

Chronaxie is defined as the time in milliseconds (duration of stimulus) when the current required to produce the aforementioned threshold contraction is twice as great as the Rheobase current.

Galvanic Tetanus Ratio (Cathodal) is the quotient obtained by dividing the value of galvanic current required to produce a sustained contraction of the muscle for the period of 1500 milliseconds by the Rheobase Current.
Repetitive Stimulation is a means of producing a sustained contraction of muscle by the use of a series of uniform galvanic stimuli having short durations separated by suitably chosen intervals.

Faradic Stimulation is the application to the muscle of the specialised wave form produced by the laboratory inductorium.

2. Instrumentation.

The instruments required for obtaining data for electrodiagnosis are:

(1) A source of galvanic current; (2) a suitable measuring device for the galvanic current; (3) a source of faradic current; (4) a stimulating electrode (percutaneous); (5) a ground or indifferent electrode; and (6) insulated wires to connect the electrodes to the sources of current. (Figure 1).

The source of galvanic current consisted of a commercial (Meditron) direct current stimulator or more specifically, a constant current impulse generator. The instrument has the following operating characteristics: (1) It is capable of delivering a stimulating impulse which is monophasic and has a rectangular wave form; (2) the duration of the impulse may be varied from 1500 milliseconds to 0.1 milliseconds in the following steps: 1500, 1000, 500, 300, 100, 60, 30, 10, 6, 3, 1, 0.6, 0.3 and 0.1 milliseconds; (3) the stimulating impulse is automatically and periodically generated by means of an electronic circuit; and (4) the interval between impulses can be varied from 1 millisecond to 4 seconds (1, 5, 12, 1000, 4000 milliseconds) by means of an interval dial.

The current output of the generator was measured by a highly sensitive, well-damped D.C. Milliammeter with full scale ranges of 400
FIGURE 1

BLOCK DIAGRAM OF ELECTRODIAGNOSIS STIMULATING APPARATUS

The source of the faradic current was a laboratory inductorium (Harvard) energized by two 1.5 volt dry cells in series.

The stimulating electrode consisted of a one-quarter inch copper rod, two inches long and rubber insulated to within one-half inch of the tip. The bare tip was covered by a piece of chamois to prevent metal-to-skin contact.
The ground or indifferent electrode consisted of a six inch strip of brass shim stock, one-half inch wide, strapped over the upper part of the leg. A piece of wet chamois slightly wider than the brass strip separated the metal from the skin of the animal.

A switching device was constructed which allowed the use of one set of electrodes for all the electrodiagnostic examinations.

3. Routine examination.
   a. Preparing the rabbit.

The anatomical area to be examined was kept free of hair by repeated careful use of a depilatory (a mixture of barium sulfide, flour, powdered castile soap and talc mixed in equal proportions).

To restrain the rabbit an assembly was constructed which consisted of a base-board, 12 inches wide by 18 inches long, with a back-board, 14 inches long and 5 inches high, fastened upright at a right angle to it. The entire apparatus is sketched in Figure 2. Adjustable elastic bands were attached to the boards to bind the rostral and caudal portions of the trunk and the ankle joint of the hind limb not under examination. The net effect was to hold the animal down onto the baseboard and back against the back-board.

The limb under examination was immobilized upon a separate table. The outline of the hind limb, when placed upon this table, was ringed by posts which position as well as restrain the limb. An adjustable elastic band secured the ankle joint. In use this table was positioned to make the animal comfortable and was then clamped to the base-board assembly. One table had
to be constructed for each side. For convenience, ground or indifferent electrodes were attached to these tables.

A piece of chamois, previously soaked in saline solution was placed across the hind limb just below the knee joint. The strip of brass shim stock was then wrapped over this chamois and the free end was then held to

FIGURE 2

RABBIT RESTRAINER
the table by a spring clip. Both the stimulating and pick-up electrodes were held in proper relation to the muscle by the familiar lead electrode holders used in physiology laboratories.

b. Procedure.

The animals were prepared for examination as previously described. The positive terminal of the stimulator was connected to the ground electrode and the negative stimulating electrode was positioned over the motor-point area. The stimulator was set to deliver a cathodal stimulus with a duration of 300 milliseconds and an interval between stimuli of 1000 milliseconds. The current control was advanced until perceptible muscle contraction could be seen. Sometimes the stimulating electrode could be shifted slightly to improve contact and perhaps yield a lower threshold reading.

When satisfactory conditions had been established (suitable threshold level, correct electrode placement, and reproducible endpoint contraction), the strength-duration curve was then run. With the interval between stimuli remaining at 1000 milliseconds, the duration of the stimuli was reduced successively, 60, 30, 10, 6, 3, 1, 0.6, 0.3 and 0.1 milliseconds. The threshold value of current required to obtain the reproducible end-point contraction was determined for each of these durations of stimuli.

The galvanic tetanus ratio with cathodal stimulation was next determined. The stimulus interval dial was set at 4000 milliseconds and the duration dial at 1500 milliseconds. This produced a one and one-half second impulse with four seconds between each impulse. The minimal current required
to give a reproducible endpoint contraction was determined. This was a quick twitch as current was made. This should correspond closely to the rheobase current found in determining the strength-duration curve. The current was then increased until a contraction was obtained where over 75 per cent of the muscle involved maintained a contraction during the entire one and one-half seconds. This was recorded as the current required for cathodal tetanus contraction. The quotient obtained by dividing the tetanus current by the simple twitch current (or rheobase current) is the galvanic tetanus ratio (cathodal).

To determine the effect of stimulating the muscle with rapidly applied repetitive stimuli, readings were made of the amount of current required to produce first a twitch contraction and then a tetanic type contraction under each of the following three conditions: Duration 1 millisecond, interval 1 millisecond (frequency 500 cps); duration 1 millisecond, interval 5 milliseconds (frequency 166 cps); and duration 1 millisecond, interval 12 milliseconds (frequency 77 cps).

Response to faradic stimulation was obtained by switching the inductorium into the stimulating circuit and the energizing its primary coil. The secondary coil was advanced toward the primary coil until the electrical output just caused the muscle to twitch. The location of this secondary coil, expressed in centimeters away from its most proximal position to the primary coil (0.3 centimeters), provides a measure of induced voltage.
Data collected during the electrodiagnosis procedure were entered into a specially prepared data sheet that was kept for each rabbit. A specimen sheet is shown in Figure 3.

5. Electromyography.

1. General considerations.

Electromyography is the recording of electrical changes which occur in skeletal muscle. An anterior horn cell in the spinal cord innervates a large number of muscle fibers through its axon cylinder, which leaves the spinal cord by way of the ventral root, reaching and entering the muscle body and eventually the myoneural junction via numerous fine nerve branches. The neuron and all muscle fibers innervated by that neuron form the biological unit of muscle function which is called a motor unit. An anatomical (named) muscle is an additive assemblage of such motor units. Under all normal conditions, a muscle fiber will be activated if it receives an impulse from the central nervous system by way of the motor nerve fiber and end-plate. Or the motor unit may be reflexly or spontaneously activated by mechanical, electrical or pharmacological stimuli to produce an electrical response. This electrical response or "action potential" may be picked up from the activated muscle by electrodes leading to an electromyograph which is capable of displaying and recording this response.

Two major criteria in electromyographic examinations are: (1) Abnormalities in the parameters of action potentials of motor units, and (2)
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<tbody>
<tr>
<td>1000 300</td>
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<tr>
<td>REPE TITIVE STIMULI</td>
</tr>
<tr>
<td>1 1</td>
</tr>
<tr>
<td>5 1</td>
</tr>
<tr>
<td>12 1</td>
</tr>
<tr>
<td>TETANUS RATIO</td>
</tr>
<tr>
<td>C.C.</td>
</tr>
<tr>
<td>C.T.C.</td>
</tr>
<tr>
<td>RHEOBASE</td>
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<tr>
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<td>FARADIC RESPONSE</td>
</tr>
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<td>CATHODE TETANUS RATIO</td>
</tr>
</tbody>
</table>

**FIGURE 3**

ELECTRODIAGNOSIS DATA SPECIMEN SHEET
the presence of fibrillation potentials. In myogenic disorders the principal contribution of electromyography is the detection of abnormal motor units. For example, the presence of low voltage, short duration, "dystrophic" potentials are characteristic of certain myopathies.

In neurogenic disorders, the principal contribution of electromyography is the detection of denervation fibrillation potentials. The subject has no control over these and they are spontaneously produced even when the muscle is at rest.

The present experiment is designed so that the control group of animals will provide a measure of the parameters of the normal "action potentials". The experimental groups of animals will provide information which can be compared or contrasted with the "normal" findings.

2. Instrumentation.

The components necessary for an electromyographic examination consist of: (1) a pickup electrode (needle type), (2) a ground electrode, (3) a pre-amplifier, (4) an audio amplifier, (5) a cathode-ray oscilloscope, and (6) a calibrating unit. (See Figure 4).

The pick-up electrode was made by inserting two No. 0 sewing needles parallel into a cork block. The needles extend from the cork for 8 mm and are 6 mm apart. After proper cleaning the needles were dipped into "radio cement" which coats the needles and acts as an insulator. Only the very tips of the needles are bare of insulation thus limiting the area of pick-up to that around the needle tips.
The electromyograph ground electrode is the same as the one described for electrodiagnosis.

The pre-amplifier, audio amplifier, and cathode-ray oscilloscope were units constructed in the department. Both the pre-amplifier and audio amplifier were push-pull, resistance-capacitance coupled amplifiers operated to accept differential signals and to reject in-phase signals. The five inch oscilloscope was of conventional design but the sweep was triggered by an external oscillator of variable frequency.
The calibrating unit consisted of an audio oscillator, a suitable voltmeter, and a calibrated attenuator which would supply known voltages having peak values from 50 microvolts to 5 millivolts. Proper selection of frequency gave convenient time base calibration in addition to amplitude calibration.

Suitable shielding to eliminate electrostatic and electromagnetic interference was provided by means of a bronze-screen cage.

A line drawing showing the electromyographic components is shown in Figure 5.
3. Routine examination.

a. Preparing the rabbit.

The animal, while still in the restraining device was transferred to the shielded cage used for making electromyographic examinations. The system ground wire was attached to the ground electrode. The bipolar needle electrode was inserted into the muscle under examination.

b. Procedure.

With the electrodes in place, the cage door was closed to completely shield the animal, and the pick-up electrode circuit was switched into the electromyograph. Occasionally following insertion of the electrode into the animal muscle, motor unit voltages, usually transient in nature, would appear. They could be abolished by quieting the animal and hence were considered to be normal postural activity. Beginning with the "quiet" or zero activity state of the muscle, action potentials were observed during various conditions of muscle functions, that is, due to voluntary effort by the animal and following mechanical stimulation to induce reflex response.

Recordings of the potentials were made by photographing the cathode-ray oscilloscope screen with a polaroid camera and by means of magnetic tape recording (which allows the recording of a continuous and approximately reproducible record). Written notes were made during the course of the examination relative to the general appearance and sound of the action potentials.
F. Pharmacological procedures.

Some of the events observed with electromyography, in particular the appearance of spontaneous fibrillation potentials in vitamin E deficient muscle, made it desirable to investigate the site of origin for these events. Various drugs were used to compare the response of E deficient and denervated muscles and to attempt to determine the source of the spontaneous potentials in the E deficient skeletal muscles.

Prostigmine was administered intramuscularly in a dose of 0.5 mg per kg.

d-Tubocurarine (200ug/kg), decamethonium and succinylcholine (100-200ug/kg) were administered intravenously for their myoneural blocking effects. These are reported to exert their effect by different mechanisms of action: d-tubocurarine as a competitive inhibitor of acetylcholine and decamethonium and succinyl choline as depolarizers of the excitable tissue membrane. Section of the sciatic nerve was done as an acute manipulation in some of the vitamin E deficient animals.

The effects of these manipulations are described in detail in Chapter III, "Results".

G. Histological studies.

Portions of skeletal muscle from the tibialis anticus, gastrocnemius, sacrospinalis, flexor and extensor muscles of the arm, and masseter were used for histological study. Bouin's fluid fixation, embedding in paraffin and
staining with hematoxylin and eosin constituted the routinely used procedure.
A silver staining technique was utilized for motor-end plate staining.

No other tissues were subjected to histological examination.

H. Summary of materials and methods.

In Table II there are listed the major groups of rabbits as they were handled in this experiment. The number of rabbits subjected to each set of conditions and a brief description of these conditions are also included.

TABLE II

GROUPS OF RABBITS USED IN THE EXPERIMENTAL PROCEDURE

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF ANIMALS</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>18</td>
<td>Control. Vitamin E deficient diet plus vitamin E (as alpha-tocopherol).</td>
</tr>
<tr>
<td>II</td>
<td>30</td>
<td>Experimental. Vitamin E deficient diet.</td>
</tr>
<tr>
<td>III</td>
<td>17</td>
<td>Chronic Denervation (unilateral).</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>Fasting for acute weight loss.</td>
</tr>
</tbody>
</table>
In Table III two columns enumerate the electrodiagnosis criteria studied and the conditions under which the electromyography observations and recordings were made.

**TABLE III**

**SUMMARY OF THE VARIOUS OBSERVATIONS MADE ON EACH GROUP OF ANIMALS**

<table>
<thead>
<tr>
<th>ELECTRODIAGNOSTIC CRITERIA</th>
<th>ELECTROMYOGRAPHIC CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Rheobase</td>
<td>A. At rest.</td>
</tr>
<tr>
<td>B. Strength-duration curves.</td>
<td>B. On animal movement.</td>
</tr>
<tr>
<td>C. Chronaxie</td>
<td>C. On reflex movement.</td>
</tr>
<tr>
<td>E. Repetitive Stimuli.</td>
<td>E. Pharmacologic procedures:</td>
</tr>
<tr>
<td></td>
<td>1. Administration of d-tubocurarine.</td>
</tr>
<tr>
<td></td>
<td>2. Administration of succinylcholine.</td>
</tr>
<tr>
<td></td>
<td>3. Administration of decamethonium.</td>
</tr>
<tr>
<td></td>
<td>4. Administration of prostigmine.</td>
</tr>
</tbody>
</table>
CHAPTER III

EXPERIMENTAL RESULTS

A. Vitamin E deficiency.

1. Gross changes.

   a. Weight changes.

   The animals gained 5 to 8 per cent in total body weight during the first two weeks on the experimental diet, with no appreciable gain during the third week. After this time there was a weight loss pattern which was the same for each individual animal but which varied in time of occurrence. In the acute stage of the deficiency, the rate of weight loss was extreme during the two to five days immediately preceding the animal's demise. The weight loss at time of death was calculated to be about 20 per cent of the total body weight determined five days prior to demise. A plot of the observed weight changes is presented in Figure 19.

   b. Physical symptoms.

   The acute stage of the deficiency lasted two to six days and terminated in death. An initial physical finding was the ease with which the rabbits could be laid on their sides, and their slowness in righting. The animals were readily pushed off their feet; those regaining an upright position were able to do so only after a violent effort. Some of the animals remained prostrated for
several days before death.

During the acute stages the animals did not partake of food apparently because they could not reach the feed and water bowls. Indeed the animals were ravenous when fed a watered version of the diet. An individual animal's existence could be protracted several days by hand feeding as learned from a prior experiment.

The most striking feature of the deficiency is the severe atrophy of the skeletal musculature present in all areas: Thigh, back and extremities.

c. Autopsy findings.

The skeletal musculature appeared atrophic, pale, and grayish in color. Subcutaneous and peritoneal adipose tissue were abundant, which would tend to indicate that the weight loss was almost entirely at the expense of the skeletal musculature. No significant changes were noted in the internal organs; Liver, spleen, kidneys, supra-renal glands, genitalia, and gastro-intestinal tract.

Animals on the control diet, which only differed from the experimental diet by the addition of alpha-tocopherol, exhibited none of the weight changes or physical symptoms (weakness, atrophy) or autopsy findings described for the experimental animals.

2. Histology.

Histological preparations were made of the skeletal muscles of vitamin E deficient rabbits. Prominent findings were:

a. Intact sarcolemma with degeneration of the contents (Hyaline degeneration).
b. Swelling of the contents of sections of individual muscle fibers and interstitial edema.

c. Loss of striations.

d. Apparent increase in number of sarcolemmal nuclei.

e. Leucocyte infiltration.

f. Prominence of connective tissue between remnants of degenerating muscle fibers.

g. Vacuolization.

The above findings are consistent with the reports on histological changes presented by previous investigators.

Photomicrographs of two tissue sections are presented in Figure 6 and 7.
FIGURE 6

PHOTOMICROGRAPH OF SKELETAL MUSCLE SECTION (TIBIALIS ANTIQUE) OF A 28 DIET DAY VITAMIN E DEFICIENT RABBIT. X400.

Explanation of letters:

A -- Aberrant attempt at regeneration.
B -- Areolar connective tissue; fibroblast nuclei; interstitial edema.
C -- Prominence of connective tissue.
D -- Fiber undergoing liquefaction and degeneration of nucleus.
E -- Granular degeneration in tapering fiber.
FIGURE 7

PHOTOMICROGRAPH OF SKELETAL MUSCLE SECTION (TIBIALIS ANTICUS) OF A 32 DIET DAY VITAMIN E DEFICIENT RABBIT.  X800.

Explanation of letters:

A  --  Area showing granular degeneration.
B  --  Other vacuolar degeneration area.
B. Electrodiagnosis.

1. Rheobase.

Rheobase values of the tibialis anticus muscles of control, vitamin E deficient, denervated and fasted animals were averaged for each of these groups as to day of measurement and are tabulated in TABLES IV, V, and VI respectively. The plots of these values are shown in FIGURE 8.

2. Strength-duration curves.

   a. Vitamin E deficiency.

      (1) Control rabbits.

      A group of six rabbits was maintained for control purposes and these rabbits were examined on the 3rd, 9th, 18th, 28th, and 48th days after entering the experiment. Vitamin E as alpha tocopherol was present in their diet. Data for the strength-duration curves were obtained from the anterior tibial muscles on both sides of each animal. Two curves for each control were thus obtained on each examination day. As there were six animals, this gave a total of twelve curves for each examination day. All these data were converted to threshold ratio values. When compared statistically, no difference was found between the curves of animals on a particular examination day and between the curves made on different days. Therefore all threshold ratio values for a particular duration of stimulus were combined and averaged. This gave a single curve with each plotted duration being the average of sixty readings (two sides x six animals x five examination days). These averaged threshold ratio values are tabulated in Table VII while the composite curve is plotted in Figure 9.
### TABLE IV

**RHEOBASE IN VITAMIN E DEFICIENCY**  
(MILLIAMPERES)

**CONTROL ANIMALS**

<table>
<thead>
<tr>
<th>Days on Diet</th>
<th>Series I</th>
<th>Series II</th>
<th>Series III</th>
<th>Series IV</th>
<th>Series V</th>
</tr>
</thead>
<tbody>
<tr>
<td>(60 sides)</td>
<td></td>
<td>(24 sides)</td>
<td>(24 sides)</td>
<td>(14 sides)</td>
<td>(6 sides)</td>
</tr>
<tr>
<td>0.84 ± .15</td>
<td>0.78 ± .15</td>
<td>0.87 ± .13</td>
<td>0.71 ± .19</td>
<td>0.84 ± .09</td>
<td>0.42 ± .09</td>
</tr>
</tbody>
</table>

**EXPERIMENTAL ANIMALS**

<table>
<thead>
<tr>
<th>Days on Diet</th>
<th>Series I</th>
<th>Series II</th>
<th>Series III</th>
<th>Series IV</th>
<th>Series V</th>
</tr>
</thead>
<tbody>
<tr>
<td>(60 sides)</td>
<td></td>
<td>(24 sides)</td>
<td>(24 sides)</td>
<td>(14 sides)</td>
<td>(6 sides)</td>
</tr>
<tr>
<td>0.84 ± .15</td>
<td>0.78 ± .15</td>
<td>0.87 ± .13</td>
<td>0.71 ± .19</td>
<td>0.84 ± .09</td>
<td>0.42 ± .09</td>
</tr>
</tbody>
</table>

### TABLE V

**RHEOBASE IN CHRONIC DENERVATION**  
(MILLIAMPERES)

**CONTROL SIDES**

<table>
<thead>
<tr>
<th>Days on Fast</th>
<th>1 day</th>
<th>4 days</th>
<th>8 days</th>
<th>18 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>(30 sides)</td>
<td>(6 sides)</td>
<td>(6 sides)</td>
<td>(6 sides)</td>
<td>(6 sides)</td>
<td>(6 sides)</td>
</tr>
<tr>
<td>0.81 ± .18</td>
<td>0.86 ± .22</td>
<td>0.87 ± .32</td>
<td>0.26 ± .16</td>
<td>0.23 ± .09</td>
<td>0.18 ± .05</td>
</tr>
</tbody>
</table>

**DENERVATED SIDES**

### TABLE VI

**RHEOBASE IN FASTING**  
(MILLIAMPERES)

<table>
<thead>
<tr>
<th>Days on Fast</th>
<th>6 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>(14 sides)</td>
<td>(6 sides)</td>
<td>(6 sides)</td>
</tr>
<tr>
<td>0.86 ± .13</td>
<td>0.77 ± .15</td>
<td>0.81 ± .10</td>
</tr>
</tbody>
</table>
(2) Experimental rabbits.

A group of 12 rabbits was used for the study of the influence of a vitamin E deficient diet. In accordance with the design of the experiment, these rabbits were examined on the days shown in Table II. Their data were recorded on the specially designed data sheets and the threshold current values were converted into threshold ratios as required for uniform handling of the

![Graph]

**FIGURE 8**

Plots of averaged rheobase values in vitamin E deficiency, denervation, and fasting.
data. Readings obtained from the examinations on the 1st to the 4th days were averaged to provide one complete average strength-duration curve which represented the behavior of the rabbits in this period from the 1st through the 4th day. The data for the examinations made on the 5th to the 11th days were also averaged and tabulated to give a representative average strength-duration curve for this period from the 5th through the 11th day. The same routine was followed in the handling of the data from examinations made in the 17th and 18th days.

After the 18th day the variance between rabbits increased so that only the examinations made upon a single day could be logically grouped together. Finally the differences between threshold ratios for the various rabbits became so great that it was no longer meaningful to average any of the data from different rabbits after the 23rd day. The average curves and the curve of an individual representative rabbit for a later day are tabulated in Table VII and are plotted in Figure 9.

In appraising the variance of the data included in any grouping, the criterion was the variance of the threshold ratios shown by the rabbits to the stimulus having a duration of 0.1 millisecond. The list of variances was as follows:

<table>
<thead>
<tr>
<th>Measurement Series</th>
<th>Diet Days</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 to 4</td>
<td>.719</td>
</tr>
<tr>
<td>II</td>
<td>5 to 11</td>
<td>.819</td>
</tr>
<tr>
<td>III</td>
<td>17 to 18</td>
<td>.496</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>.992</td>
</tr>
<tr>
<td>V</td>
<td>23</td>
<td>1.416</td>
</tr>
</tbody>
</table>
### TABLE VII

**STRENGTH-DURATION DATA OF VITAMIN E DEFICIENCY (THRESHOLD RATIOS)**

<table>
<thead>
<tr>
<th>CONTROL ANIMALS</th>
<th>EXPERIMENTAL ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (Millisec)</td>
<td>Series I</td>
</tr>
<tr>
<td>300</td>
<td>1.00</td>
</tr>
<tr>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>60</td>
<td>1.00</td>
</tr>
<tr>
<td>30</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>1.00</td>
</tr>
<tr>
<td>5.6</td>
<td>1.11</td>
</tr>
<tr>
<td>3</td>
<td>1.28</td>
</tr>
<tr>
<td>1</td>
<td>1.54</td>
</tr>
<tr>
<td>0.6</td>
<td>1.79</td>
</tr>
<tr>
<td>0.3</td>
<td>2.32</td>
</tr>
<tr>
<td>0.1</td>
<td>3.60</td>
</tr>
</tbody>
</table>

Interval between stimuli always 1000 milliseconds.
FIGURE 9

STRENGTH-DURATION CURVES IN VITAMIN E DEFICIENCY

b. Chronic denervation.

Six rabbits unilaterally denervated were examined in accordance with the design of the experiment and their data were recorded on the Electrodiagnosis data sheets.
(1) Control side.

Considering the normal or control side first, all six values of threshold ratio for each duration of stimulus, on the first day's examinations, were averaged. Their standard deviation was computed and recorded. This was done for all of the respective stimuli used and the averages were charted to form one composite strength-duration curve for all six rabbits for the first day. As the days went by and the succeeding examinations were made, the same process was repeated and the resulting curves were compared. It was apparent at the close of the periods of examinations that the readings for the specific days were essentially alike and that they could be averaged. For that reason all of the data resulting from the application of a given stimulus to each one of the rabbits during the period of study were averaged and the standard deviation was computed by the long method. These averages are tabulated in Table VIII under the heading "Control Side". The curve representing these data has been plotted in Figure 10.

(2) Denervated side.

Considering the denervated side of the rabbits, there were examinations on the first post-operative day and also on the fourth, eighth, eighteenth and thirtieth days. The six threshold ratios for each duration of stimulus, on a given day, were averaged and the standard deviation of the group was computed. These are tabulated on Table VIII for each examination. The five average curves are plotted in Figure 10.
# Table VIII

**Strength-Duration Data of Chronic Denervation**

<table>
<thead>
<tr>
<th>Duration (Milliseconds)</th>
<th>Control Side</th>
<th>Denervated Side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Day</td>
<td>4 Days</td>
</tr>
<tr>
<td>300</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>100</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>60</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>30</td>
<td>1.01</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**T.R. (Threshold Ratio)**

S.D. (Standard Deviation)

Interval between stimuli always 1000 milliseconds.
FIGURE 10

STRENGTH-DURATION CURVES IN CHRONIC DENERVATION

c. Fasting study.

A group of seven rabbits were subjected to acute fasting by being maintained on a normal vitamin-mineral diet but one which was low in calorie
value and essentially devoid of bulk. In keeping with the design of the
experiment, electrodagnostic examination was made on the tibialis anticus
muscles on the day the animals entered into the experiment and again when the
desired end-point, loss of approximately 20 per cent of initial total body
weight, was reached. This occurred after six days. These seven animals were
examined bilaterally which gave 1/4 curves in all for the initial examination
and 1/4 curves for the end-point examination. These data were converted to
threshold ratios and were then averaged for each stimulus duration giving one
composite curve for each day.

Three animals were kept on this experiment for an additional six
days for a total of fourteen days on the fasting diet. This represented over
twice the time during which the vitamin K deficient animals exhibited their
weight loss. The average weight loss of the fasting animals was 32 per cent of
the initial body weight after fourteen days.

All the curves with their associated standard deviations are in
Table IX. These curves were not significantly different from the Vitamin K
Control strength-duration curve (Figure 9), hence they were not plotted.

3. Chronaxie.

Chronaxie values, in all cases, were obtained from the plotted
strength-duration curves. Having obtained the chronaxie values in this manner,
the values of a particular group of animals for a particular day were combined
and the average values computed. The standard deviations of these average
values were then estimated.
### TABLE IX

**STRENGTH-DURATION DATA OF FASTING STUDY**

*(THRESHOLD RATIOS)*

<table>
<thead>
<tr>
<th>Duration (Milli-seconds)</th>
<th>0 days (14 sides)</th>
<th>6 days (14 sides)</th>
<th>14 days (6 sides)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>100</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>60</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>50</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>1.08</td>
<td>1.06</td>
<td>1.10</td>
</tr>
<tr>
<td>3</td>
<td>1.35 ± .132</td>
<td>1.20 ± .165</td>
<td>1.24 ± .143</td>
</tr>
<tr>
<td>1</td>
<td>1.65 ± .210</td>
<td>1.70 ± .214</td>
<td>1.52 ± .217</td>
</tr>
<tr>
<td>0.6</td>
<td>1.80 ± .301</td>
<td>1.90 ± .310</td>
<td>1.76 ± .305</td>
</tr>
<tr>
<td>0.3</td>
<td>2.20 ± .416</td>
<td>2.20 ± .404</td>
<td>2.27 ± .369</td>
</tr>
<tr>
<td>0.1</td>
<td>3.50 ± .530</td>
<td>3.32 ± .575</td>
<td>3.43 ± .474</td>
</tr>
</tbody>
</table>

*3. D. (Standard Deviation)*

Interval between stimuli always 1000 milliseconds.
The data are presented in tabular form as follows: Vitamin E Study (Control and Experimental animals), Table X, Chronic Denervation Study (Control and Denervated sides), Table XI; and the Fasting Study, Table XII.

A summary of the data from all of the studies is graphically represented in Figure 11. A plot of the chronaxie distribution of individual vitamin E deficient animals appears in Figure 12.
## TABLE X

**CHRONAXIE OF VITAMIN E DEFICIENCY**

**MILLISECONDS**

<table>
<thead>
<tr>
<th>CONTROL ANIMALS</th>
<th>EXPERIMENTAL ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days on Diet</td>
<td>Days on Diet</td>
</tr>
<tr>
<td>Series I</td>
<td>Series II</td>
</tr>
<tr>
<td>1-4 Days</td>
<td>5-11 Days</td>
</tr>
<tr>
<td>464 ± 157</td>
<td>478 ± 171</td>
</tr>
</tbody>
</table>

## TABLE XI

**CHRONAXIE OF CHRONIC DENERVATION**

**MILLISECONDS**

<table>
<thead>
<tr>
<th>CONTROL SIDES</th>
<th>DENERVATED SIDES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days After Denervation</td>
<td>Days After Denervation</td>
</tr>
<tr>
<td>1 Day P.O.</td>
<td>4 Days P.O.</td>
</tr>
<tr>
<td>349 ± 155</td>
<td>382 ± 180</td>
</tr>
</tbody>
</table>

## TABLE XII

**CHRONAXIE IN FASTING**

**MILLISECONDS**

<table>
<thead>
<tr>
<th>CONTROL</th>
<th>6 Days</th>
<th>14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days on Fast</td>
<td>Days on Fast</td>
<td></td>
</tr>
<tr>
<td>420 ± 141</td>
<td>460 ± 139</td>
<td>440 ± 192</td>
</tr>
</tbody>
</table>

S. D. (Standard Deviation)
No significant differences between ratio values for a particular day were found.

divided by the standard value for that particular examination, and (2) where

the procedure employed for studying the repetitive stimulation data

were
derived data representing the relative stimulation due

EXPERIMENTAL

VALUES IN VITAMIN E

SHOW OF INDIVIDUAL CHRONAXIES

PLOTTED AS

TIME (DAYS)

0

30

20

10

0

40

CHRONAXIE (MILLISECONDS)

E244

E245

E241

E240, E242, E243

E251, E248

E249, E247, E250
the ratios were combined and averaged. The data for the particular groups of animals are presented in the following tables and figures: Vitamin E Study (Control and Experimental Animals) Table XIII, Figure 13; Chronic Denervation Study (Control and Denervated Sides) Table XIV, Figure 13; and the Fasting Study, Table XV. Because the data from the Fasting Study are essentially similar to the Vitamin E Control animals, (Figure 13) they were not plotted.

5. Galvanic to amus ratio.

The galvanic tetanus ratios (cathodal) were obtained by dividing the current required for cathodal tetanus contraction by the current required for simple twitch contraction (Rheobase). These data were then combined as to particular measurement day and particular animal group and then averaged. Standard deviations were estimated by the long method. All the data are presented in the following tabular sections: Vitamin E Study (Control and Experimental Animals) Table XVI; Chronic Denervation Study (Control and Experimental Sides) Table XVII and the Fasting Study, Table XVIII. The data from all the groups are plotted in Figure 14.

6. Faradic stimulation.

Recordings of skeletal muscle response to faradic stimulation were obtained as described in "Materials and Methods".

No difference in type of response or in threshold to the faradic stimulation found for the following groups of animals: vitamin E controls, vitamin E experimental animals, the control sides of the denervated animals, and the fasting animals. All responded with secondary coil set at 11.0 centimeters.
TABLE XIII
REPEITIVE STIMULATION IN VITAMIN E DEFICIENCY
(THRESHOLD RATIOS)

<table>
<thead>
<tr>
<th>FREQUENCY</th>
<th>CONTROL ANIMALS</th>
<th>EXPERIMENTAL ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Series I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-4 Days</td>
</tr>
<tr>
<td>500 cps</td>
<td>1.48 ± .100</td>
<td>1.67 ± .108</td>
</tr>
<tr>
<td>166 cps</td>
<td>1.34 ± .511</td>
<td>1.62 ± .370</td>
</tr>
<tr>
<td>77 cps</td>
<td>1.34 ± .507</td>
<td>1.61 ± .362</td>
</tr>
</tbody>
</table>

TABLE XIV
REPEITIVE STIMULATION IN CHRONIC DENERVATION
(THRESHOLD RATIOS)

<table>
<thead>
<tr>
<th>FREQUENCY</th>
<th>CONTROL SIDES</th>
<th>EXPERIMENTAL SIDES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 Day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P.O.</td>
</tr>
<tr>
<td>500 cps</td>
<td>1.63 ± .418</td>
<td>1.41 ± .507</td>
</tr>
<tr>
<td>166 cps</td>
<td>1.38 ± .244</td>
<td>1.34 ± .477</td>
</tr>
<tr>
<td>77 cps</td>
<td>1.37 ± .246</td>
<td>1.43 ± .497</td>
</tr>
</tbody>
</table>

S. D. (Standard Deviation)
### TABLE XV

**REPEITIVE STIMULATION IN FASTING**  
*(THRESHOLD RATIOS)*

<table>
<thead>
<tr>
<th>FREQUENCY</th>
<th>EXPERIMENTAL ANIMALS</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days on Fast</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 Days</td>
<td>14 Days</td>
</tr>
<tr>
<td>500 cps</td>
<td>1.42 ± .211</td>
<td>1.52 ± .272</td>
</tr>
<tr>
<td>166 cps</td>
<td>1.29 ± .345</td>
<td>1.41 ± .158</td>
</tr>
<tr>
<td>77 cps</td>
<td>1.29 ± .348</td>
<td>1.40 ± .164</td>
</tr>
</tbody>
</table>

S.D. (Standard Deviation)
Figure 13

Plot of repetitive stimulation values in vitamin E deficiency and chronic denervation.
TABLE XVI

GALVANIC TETANUS RATIO IN VITAMIN E DEFICIENCY

<table>
<thead>
<tr>
<th>Control Animals</th>
<th>Experimental Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days on Diet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-4 Days</td>
</tr>
<tr>
<td></td>
<td>Series I</td>
</tr>
<tr>
<td>Aver. S.D.</td>
<td>(60 sides)</td>
</tr>
<tr>
<td>Aver. S.D.</td>
<td>3.20 ± 1.87</td>
</tr>
</tbody>
</table>

TABLE XVII

GALVANIC TETANUS RATIO IN CHRONIC DENERVATION

<table>
<thead>
<tr>
<th>Control Sides</th>
<th>Days After Denervation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Day</td>
</tr>
<tr>
<td>Aver. S.D.</td>
<td>3.35 ± .694</td>
</tr>
</tbody>
</table>

TABLE XVIII

GALVANIC TETANUS RATIO IN FASTING

<table>
<thead>
<tr>
<th>Control Days on Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>(6 Days)</td>
</tr>
<tr>
<td>(14 sides)</td>
</tr>
<tr>
<td>3.20 ± .175</td>
</tr>
</tbody>
</table>

S. D. (Standard Deviation)
For the denervated side of the chronic denervation animals a continually increasing threshold after four days required that the secondary coil be moved.
toward the primary coil so as to effect muscle contraction by a greater electrical output. After thirty days, response to faradic stimulation could still be elicited with a secondary coil setting of 9.0 centimeters. After sixty days, a response to a secondary coil setting of 8.0 centimeters could be obtained.

C. Electromyography.

1. Potentials at rest.
   a. Vitamin E control animals.

   Of fifteen control animals examined, "electrical silence" was the consistent finding. In no case was any fibrillation-like potential seen.

   b. Vitamin E deficient animals.

   Thirty animals were examined during the course of this study. After ten to fifteen days, transient (10 to 20 seconds total duration) "irritation" type potentials were evoked following insertion of the needle electrodes. These potentials may be characterized as very short duration (0.5 millisecond) low amplitude (less than 50 microvolts) potentials. At fifteen to twenty days, potentials were recorded at rest which were of short duration (1 to 2 milliseconds) and low amplitude (50 to 250 microvolts) seen in Figure 15. These were persistent, spontaneous "fibrillation" type potentials. They were continually firing, though asynchronously. They are further characterized by the sharp, high frequency sounds which they produce, in all muscles and all these animals.

   c. Chronic denervation animals.

   The tibialis anticus muscles of unilaterally denervated animals were examined electromyographically daily during the first four days following
FIGURE 15

POTENTIALS AT REST

Explanation of Figure:

A -- Normal muscle at rest ("electrical silence")
B -- Vitamin E deficiency. Insertion activity (non-persisting) after 12 days.
C -- Vitamin E deficiency. Spontaneous fibrillation-like activity (persisting) after 15 days.
D -- Chronic Denervation. Insertion activity (non-persisting) after 12 hours.
E -- Chronic Denervation. Denervation fibrillation potentials after 4 days.
F -- Chronic Denervation. Increased fibrillation activity after 8 days.
surgery and on the eighth, eighteenth, and thirtieth days respectively. Prior to surgery, the animals were examined and electrical silence was the finding at rest. Three hours after surgery, electrical silence still prevailed. At twelve hours after surgery, non-persisting "irritation" potentials were elicited. These were also found on the second and third days. On the fourth day, persistent fibrillation potentials of denervation were recorded. These potentials had the following parameters: 1 to 2 milliseconds duration, 50 to 250 microvolts amplitude, and appeared as monophasic and diphasic spikes. (Figure 15). Characteristically, they were spontaneous and persistent in nature, and produced sharp, high frequency sounds. On the eighth day, there was an increased number of fibrillation potentials being fired. On the eighteenth and thirtieth days the fibrillation potentials continued while at complete rest. This has been described as a "base-line fibrillation activity".

In contrast, the anterior tibial muscle of the opposite non-denervated side did not produce any potentials, that is, electrical silence was the finding at rest. No irritation potentials were elicited on needle insertion; no fibrillation type potentials were seen at any time.

d. Fasting animals.

The seven animals subjected to starvation were examined on the first and sixth days. At rest, the anterior tibial, gastrocnemius, sacrospinalis, masseter and flexor and extensor muscles (bilaterally) of the foreleg exhibited normal base-line quiet at the beginning and end of the starvation period. The three animals continued on the fasting diet were found to have the same normal electromyographic findings at the end of fourteen days.
2. Potentials due to movement.

a. Vitamin E control animals.

Examination of the series of muscles previously described produced the following results: On activity where just a few motor units were produced, the parameters were observed to be 4 to 6 milliseconds in duration and 500 to 1,000 microvolts in amplitude, with wave forms usually appearing biphasic, and occasionally triphasic; with activity where many motor units were produced, the picture was that of an "interference pattern" in which so many motor units were firing as to make any one motor unit indistinguishable. The peak amplitude was observed to exceed one millivolt.

b. Vitamin E deficiency animals.

Motor units persisted during the entire period of experimental observation, that is, it was always possible for the animal to produce electromyographically visible motor units even during the acute stages of the nutritional muscular dystrophy though muscle contraction was not always visible grossly.

The parameters of the motor unit response were observed to change during the course of the experimental procedure. During the first twenty days the motor units appeared normal with normal parameters: 4 to 6 milliseconds duration, 400 to 1,000 microvolts in amplitude. The wave form and sound were essentially normal. Sustained "interference" patterns had peak amplitudes of above 1 millivolt.

After 20 days, there was a diminution in amplitude and a shortening of duration of the motor units elicited.
At the final stages of the nutritional muscular dystrophy, voluntary contractions of the muscle produced short duration bursts (100 milliseconds total duration) of very high frequency potentials, reaching peaks of 200 to 400 microvolts.

The previously described potentials due to movement are illustrated in Figure 16.

3. Pharmacological findings with neuromuscular drugs.

To obtain statistical significance, all of the following manipulations were tested on a minimum of seven animals.

a. d-Tubocurarine.

In the normal animal, intravenous administration of 150-200 ug/kg of d-tubocurarine resulted in the cessation of motor unit activity with a paralysis of the animal. In vitamin E deficient animals, intravenous administration of the same dose of d-tubocurarine resulted in cessation of motor unit activity with paralysis but there was no effect on the presence of the spontaneous fibrillation potentials. When larger doses were administered (300 to 400 ug/kg), respiratory paralysis was produced necessitating artificial respiration, but there was no effect on the fibrillation potentials. If respiration was not maintained resulting in the death of the animal, the fibrillation potentials continued to fire even after three hours. d-Tubocurarine in full blocking doses (300 ug/kg) had no effect on the fibrillation potentials of the denervated animals.

b. Effect of succinylcholine and decamethonium.

In the normal animal, intravenous administration of succinylcholine
FIGURE 16

POTENTIALS DUE TO MOVEMENT

Explanation of Figure:

A -- Normal muscle. Single motor unit.
B -- Normal muscle. Many motor units firing; "interference pattern".
C -- Vitamin E deficiency. 18 days. Many motor units; "interference pattern".
D -- Vitamin E deficiency. 26 days. Movement produces bursts of low amplitude, short duration potentials.
In the normal animal, intravenous administration of succinylcholine (200 ug/kg) produces a "shower of activity" which has been described as a "wave of depolarization". This was seen in the animals for about two minutes following which all activity ceased. Motor unit activity began to return after ten to fifteen minutes.

In the vitamin E deficient animal as well as in the denervated animal, a characteristic "shower of activity" followed intravenous administration of 200 ug/kg of succinylcholine. In both groups of animals, however, all electrical activity ceased after about one to two minutes, both the motor units of the vitamin E animals and the fibrillation potentials of the E deficient and denervated animals. The fibrillation in both animals was seen to return after about 10 minutes; fibrillation potentials always preceded the return of motor unit activity in the E deficient animals. These findings are illustrated in Figure 17.

Decamethonium (150 to 200 ug/kg) was administered to three each of denervated and vitamin E deficient animals. Events identical to those described above for succinylcholine were also seen following decamethonium.

c. Effect of prostigmine (Acostigmine).

In normal animals, prostigmine administered intramuscularly, 0.5 mg/kg, resulted in the augmentation of the motor unit potentials. Minimal effort by the animal resulted in prolonged bursts of motor unit activity, usually with an increase in amplitude.

In the vitamin E deficient animals, five minutes after the administration of prostigmine intramuscularly, there was an augmentation of motor unit
FIGURE 17

EFFECT OF SUCCINYLCHOLINE ON THE SPONTANEOUS POTENTIALS FROM MUSCLES OF VITAMIN E DEFICIENT AND DENERVATED ANIMALS

Explanation of Figure:

Controls.
- A -- Muscle at rest. Electrical silence.
- B -- Wave of activity immediately following succinylcholine administration.
- C -- Electrical silence after 2 minutes.
- D -- Return of motor unit activity after 12 minutes.

Vitamin E Deficiency.
- E -- Muscle at rest. Spontaneous potentials present.
- F -- Wave of activity immediately following succinylcholine.
- G -- Electrical silence after 2 minutes.
- H -- Return of activity after 10 minutes.

Chronic Denervation.
- I -- Muscle at rest. Denervation fibrillation potentials present.
- J -- Wave of activity immediately following succinylcholine.
- K -- Electrical silence after 2 minutes.
- L -- Return of activity after 14 minutes.
activity as seen in the normal rabbits. The increase in motor unit activity has the effect of "masking" any changes in the fibrillation potentials. When the sciatic nerve was severed, the motor unit activity ceased. However, it was then possible to detect an increase in number and frequency of discharge of the fibrillation potentials.

In the chronically denervated animals, the administration of prostigmine resulted in an increase in amplitude and in the frequency of the fibrillation potentials.

The effects of prostigmine on the electromyographic recordings of the potentials of normal, vitamin E deficient, and chronically denervated animals are illustrated in Figure 16.

D. Summary of electrodiagnostic, electromyographic, and pharmacologic Findings

Summaries of the findings in the electrodiagnosis, electromyography, and response to neuromuscular drugs of normal, vitamin E deficient, and chronically denervated animals have been prepared and are presented in Table XIX, XX and XXI respectively. Comments on findings in the normal (control) animals are also applicable to the fasted animals.
FIGURE 16
EFFECT OF PROSTIGMINE ON CONTROL VITAMIN E DEFICIENCY AND CHRONIC DENERVATION POTENTIALS

Explanation of Figure:

Control.
A -- Before prostigmine. Movement produces motor unit activity.
B -- 5 minutes after prostigmine. Movement produces sustained activity.

Vitamin E Deficiency.
C -- Before prostigmine. Spontaneous activity at rest.
D -- 5 minutes after prostigmine. Increased spontaneous activity at rest.

Chronic Denervation.
E -- Before prostigmine. Fibrillation potentials present.
F -- 5 minutes after prostigmine. Increased number of fibrillation potentials.
TABLE XIX
SUMMARY OF ELECTRODIAGNOSTIC FINDINGS

<table>
<thead>
<tr>
<th></th>
<th>Control Animals</th>
<th>Vitamin E Deficient Animals</th>
<th>Chronic Denervation Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RHEOBASE</strong></td>
<td>Normal, no change with time.</td>
<td>Normal with slight decrease toward end.</td>
<td>Decreases as denervation continues.</td>
</tr>
<tr>
<td><strong>STRENGTH-DURATION CURVES</strong></td>
<td>No change in shape with time.</td>
<td>Upward trend of curves as the deficiency continues (more stimulation required to produce a contraction); departure from rheobase occurs at stimuli of longer and longer duration; curves show discontinuities (tendency to plateau).</td>
<td>Marked upward trend of curves as the denervation continues; departure from rheobase occurs at stimuli of longer and longer duration; curves show no discontinuities.</td>
</tr>
<tr>
<td><strong>CHRONAXIE</strong></td>
<td>Normal, no change with time.</td>
<td>Early—no change; late, with onset of deficiency symptoms, there is a rapid increase in chronaxie.</td>
<td>Rapid continuing increase in chronaxie immediately following denervation.</td>
</tr>
<tr>
<td><strong>REPE TITIVE STIMULATION</strong></td>
<td>Normal pattern; no change with time.</td>
<td>Slight increase in threshold and change in pattern.</td>
<td>Marked increase in threshold and change in pattern.</td>
</tr>
<tr>
<td><strong>GALVANIC TETANUS RATIO (CATHODAL)</strong></td>
<td>Within normal limits; no change with time.</td>
<td>Slow initial increase; rapid later.</td>
<td>Immediate and rapid increase during eight days; slowly and continually decreasing until thirtieth day.</td>
</tr>
<tr>
<td><strong>FARADIC STIMULATION</strong></td>
<td>Always positive findings. No change with time.</td>
<td>Always positive findings. No change with time.</td>
<td>No change initially.</td>
</tr>
</tbody>
</table>
TABLE XX

SUMMARY OF ELECTROMYOGRAPHIC FINDINGS

<table>
<thead>
<tr>
<th></th>
<th>Control Animals</th>
<th>Vitamin E Deficient Animals</th>
<th>Chronic Denervation Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>POTENTIALS AT REST</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical silence</td>
<td></td>
<td>Electrical silence to</td>
<td>Denervation fibrillation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fifteen days on diet;</td>
<td>potentials appear on the</td>
</tr>
<tr>
<td></td>
<td></td>
<td>after this, spontaneous</td>
<td>fourth day following</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fibrillation-like</td>
<td>surgery.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>potentials to death.</td>
<td>Observed up to thirty days.</td>
</tr>
<tr>
<td><strong>POTENTIALS DUE TO MOVEMENT</strong></td>
<td>Normal.</td>
<td>Diminution in amplitude</td>
<td>None.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>of motor unit potentials;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>decrease in number of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>motor units responding;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>presence of &quot;dystrophic&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>potentials in the acute</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(final) stage of the</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>deficiency.</td>
<td></td>
</tr>
</tbody>
</table>

TABLE XXI

SUMMARY OF PHARMACOLOGICAL FINDINGS

<table>
<thead>
<tr>
<th></th>
<th>Control Animals</th>
<th>Vitamin E Deficient Animals</th>
<th>Chronic Denervation Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>d-TUBOCURARINE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical silence</td>
<td></td>
<td>No motor units; spontaneous</td>
<td>Fibrillation potentials</td>
</tr>
<tr>
<td></td>
<td></td>
<td>potentials continue.</td>
<td>continue.</td>
</tr>
<tr>
<td>**SUCCEINYL-CHOLINE AND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DECAESTRONIUM</td>
<td></td>
<td>Electrical silence.</td>
<td>Electrical silence.</td>
</tr>
<tr>
<td><strong>PROSTIGIMINE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical silence at rest;</td>
<td></td>
<td>Increase in number of</td>
<td>Increase in number of</td>
</tr>
<tr>
<td>on movement, increased</td>
<td></td>
<td>motor units and voltage</td>
<td>fibrillation potentials</td>
</tr>
<tr>
<td>voltage.</td>
<td></td>
<td>of motor units. Increase in</td>
<td>firing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>number of spontaneous</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>potentials.</td>
<td></td>
</tr>
</tbody>
</table>
IV DISCUSSION

A. Findings established by this investigation.

1. Electrophysiology.
   a. Electrodiagnosis.

(1) Results of the present investigation.

Comparison of the data from skeletal muscles of vitamin E deficient animals with the data obtained from skeletal muscles of (1) control animals on the same diet with vitamin E, (2) denervated animals maintained on the control diet, and (3) acutely fasted animals on a low caloric diet which contained vitamin E, indicates rather conclusively that a similarity exists between the late stages of the vitamin deficiency (18-26 days) and the early period of denervation (4-8 days). Fasting per se did not yield any findings which differed from the control data.

Discussing each electro-diagnostic procedure in turn:

Rheobase in E deficiency is seen to decrease with a slope comparable to the denervation curve with the time relationship described above.

Strength-duration curves of E deficiency showed the same upward trend as denervated muscle (Figures 9 and 10) indicating decreasing excitability in the tissue. In addition, between the durations of 1.0 and 10.0 milliseconds, the vitamin E curves depart from a smooth or “fair” characteristic and at 3.0
milliseconds a discontinuity appears to be developing in curves on successive days. In other words, this sharp change of direction becomes more prominent as the number of days on the diet increases. A discontinuity is reported by previous investigators as being present in muscle partly denervated, in very early denervation, and when nerve regeneration is taking place following re-establishment of nerve supply to completely denervated muscle (Adrian, 1916; Pollock et al., 1925). In view of this, the finding of a discontinuity in this investigation was considered to be an important finding. In order to find out whether this departure from a smooth curve should be attributed to something other than chance, it was decided that a statistical examination of the data in the region of 3.0 milliseconds should be made.

Considering the curve for "20 days" in Figure 9, we observe that each point was an average of 14 observations. If we then examine the data for the points plotted at duration of 1.0, 3.0, 5.5, and 10 milliseconds, we can compute the sum of squares of the observations about their respective means. We note further that the number of degrees of freedom available for estimating the standard error of these data is 52. The total sum of squares is 5.2321 units and the standard error of the mean of 14 observations is .0848 units. From a smooth curve drawn from the data for this 20 day characteristic, ignoring the data for durations of 3.0 and 5.5 ms., we can read an ordinate of 1.61 units at the duration of 3.0 ms. This enables us to set up Student's "t" test to determine the probability of finding an average of 14 observations at an ordinate of 1.61 units and at a duration of 3.0 ms.
Then:  
\[
t = \frac{1.81 - 1.61}{0.0848} = 2.36
\]

According to a table of probabilities associated with Student's distribution for 52 degrees of freedom, the probability of finding a deviation from a fair curve as great as we observe here purely by chance is less than .01.

The same evaluation was made of the 23 day E deficiency strength-duration curve. It was found that

\[
t = \frac{2.55 - 2.38}{0.1205} = 2.24
\]

According to the "t" table the probability is less than .025 and is therefore significant. The fact that both curves displayed this significant discontinuity, and in the same direction, is taken to be incontrovertible evidence for the reality of the discontinuity.

The averaged chronaxie values of the denervated muscles were found to rise from a normal value of 0.35 milliseconds obtained on the first day to 7.35 milliseconds in four days with an upper limit of 30.5 milliseconds at the end of thirty days. The vitamin E deficient animals exhibited a normal or control chronaxie at the times of the first examinations period (1-4 days) and the second examination period (8-11 days). By the third period (17-18 days) the chronaxie was found to have risen to 1.12 milliseconds, and at the end of 23 days to 5.45 milliseconds. In those animals surviving beyond this time (up to 40 days) the upper limit of chronaxie reached was 10.2 milliseconds. Figure 10, a comparative plot of the average chronaxie values in normal, denervated, and vitamin E deficient muscle, again shows the parallelism between the E deficient and denervated muscles.
Ratio values obtained for all three frequencies of repetitive stimulation used (500, 166, and 77 cycles per second) in normal or control muscles appeared to be equal.

In the unilaterally denervated animals, the repetitive stimuli ratio values for the denervated muscles on the first day following denervation were normal. By the fourth day, there was a statistically significant increase in the ratio values for all frequencies. On the eighth day the ratio values continued to increase and it was found that more current was required to produce a response with a frequency of 77 cps, less for the 166 cps frequency, and still less for the 500 cps frequency. There is a statistically significant difference between each of the three frequencies and for all three from the normal or control values. The eighteenth and thirtieth days saw a further increase in ratio values with the frequency responses arranged in the same order as on the eighth day. (Figure 13).

The ratio values of the vitamin E deficient animals were normal for the first ten days. There was a slight elevation in ratio values to the twentieth day. All the frequencies participated in this increase with no difference found between them. Beginning with the twentieth day, all frequency ratio values were seen to rise sharply. (Figure 13). The significant findings in the responses to repetitive stimulation are: (1) the rapidly increasing ratio values in the denervated muscles for all frequencies, with a statistical significance between ratio values for all frequencies when the muscle is "completely" denervated, and (2) that the slope of the vitamin E deficient
curves from the twentieth to the twenty-fourth day have a degree of similarity
to the curves obtained during the first four days of denervation.

In the present investigation, the results of examination for galvanic
tetanus ratio (Figure 14) reveal the following significant features: Normal or
control muscles yield ratio values of the range 3.1 to 3.5. Denervated muscles
show a rapid initial increase in galvanic tetanus ratio from 3.5 to a peak of
6.5 during the first eight days, and a slow but progressive decrease to 5.7 over
the next twenty-two days, the end-point of the experimental procedure. Muscles
of the fasted animals yielded ratio values within the normal or control range.
The galvanic tetanus ratio values of the muscles of the vitamin E deficient
animals revealed a slow initial increase for the first eighteen days followed
by a rapid increase to 8.2 at the twenty-fourth diet day.

In the present investigation, no difference in type of response or in
threshold to faradic stimulation was found for the vitamin E controls, vitamin
E experimentals, control sides of the denervated animals, and the fasting
animals. In all muscles examined, the threshold current was obtained with the
secondary coil of the inductorium set at 11.0 centimeters.

For the denervated side of the unilaterally denervated animals,
excitability of the muscle to faradic current decreased, responding with a
secondary coil setting of 10.0 centimeters at four days, 9.0 centimeters at
30 days, and 8.0 centimeters at 60 days.

Faradic stimulation appears to be the only electrodiagnostic test
employed in this investigation where the vitamin E deficient muscle did not
respond at some point with the findings for the denervated muscle. This is
not considered a striking finding because:

(1) The response of denervated muscle to percutaneous faradic stimulation did persist for the entire time of the investigation (60 days).

(2) The decrease in faradic stimulation threshold of the denervated muscle was minimal when compared with the other electrodiagnostic criteria.

(3) The method of measuring the faradic stimulation by measuring the setting of the secondary coil is at best very crude.

(2) Comparison of electrodiagnostic findings in vitamin E deficiency with the findings of earlier investigators.

As pointed out in the introduction, the only work which appears in the literature relating any of the EDE criteria used in this investigation to E deficient skeletal muscle was that of Victor (1934). He studied rheobase and chronaxie, and reported that both tended to rise in muscle of vitamin E deficient rabbits.

Our chronaxie findings are in agreement with those reported by Victor. However, rheobase in the E deficient rabbits in our investigation was seen to decrease. The difference obtained in rheobase may be accounted for by that fact that Victor used direct muscle stimulation with needle electrodes, whereas percutaneous electrodes were used in this investigation. In addition, he used a Condenser Discharge Apparatus for stimulation; we used a constant current impulse stimulation which puts out a controlled "square wave" of galvanic current. Finally, Victor based his results on single measurements taken from many different animals. Our findings are based on many measurements for each animal and for many different animals.
(3) Comparison of electrodiagnosis findings in denervation with the findings of earlier investigators.

In addition to allowing a comparison to be made with E deficiency findings, the denervation findings of this investigation also provide data which can be used to re-evaluate electrodiagnosis findings of previous investigators.

The electrodiagnosis findings in denervation in this investigation are in general agreement with those of earlier workers as regards strength-duration curves, chronaxie, and repetitive stimulation.

In the areas of controversy, rheobase, galvanic tetanus ratio faradic stimulation, the position taken based on the data obtained in this work is as follows:

In rheobase, it appears that with percutaneous stimulation and under well controlled experimental conditions, it is possible to demonstrate a decrease in rheobase values.

In galvanic tetanus ratio, it has been reported by previous workers (particularly the Pollock group) that GTR values in denervation approach unity. They obtained GTR values of 1.5 in cats after 15 to 20 days of denervation.

In our data, at no time did the GTR approach 1.0. Moreover, examination of our data (Figure 14) shows that after an initial rise, the "trend" of the GTR curve in denervation appears to be downward but even after 30 days has not reached the control levels much less unity. While Pollock used cats, and rabbits were used here, species differences are not believed to account for the
differences in the data. The number of cats used to arrive at the Pollock data is not mentioned in their report, but one of the investigators admits (verbally that "several animals" were used. But this is probably not a factor either, for at no time in the present experiments, did the GTR approach 1.0. Even though we are not in agreement with earlier investigators (specifically the Pollock group) our contribution to knowledge of galvanic tetanus ratio findings is as follows:

1. Under controlled experimental conditions and with a statistically significant number of animals, it is possible to demonstrate that galvanic tetanus ratio values for normal anterior tibial muscles of rabbits fall within a limited range (3.1 to 3.5). Pollock reported 3.5 to 6.0 for gastrocnemius muscles of cats.

2. In denervation of the anterior tibial muscles of rabbits, the early galvanic tetanus ratio curve is marked by a rapid initial increase followed by a progressive galvanic tetanus ratio decline. The character of this early initial increase has not been previously described in the literature.

In skeletal muscle response to faradic stimulation, the results of the experimental denervation of this investigation compare with the experimental findings of Erb and the clinical findings of Roberts (1916), Hayfield and his co-workers and Pollock et al in that there is decreased excitability of denervated muscle to faradic stimulation. Where the present results differ is in the reported "loss" of response to faradic percutaneous stimulation fifteen days after denervation. In our findings, response of denervated muscle to
percutaneous faradic stimulation is only slightly diminished even after 60
days. This corresponds to Erb's finding that on direct stimulation of denerv-
ated muscle there is a limited contraction for a long time after injury and
with Pollock's finding in the cat that excitation by faradic stimulation per-
sists throughout the period of denervation both when the muscle is stimulated
percutaneously and directly.

b. Electromyography.

(1) Results of the present investigation.

In the vitamin E control animals, in the fasted animals, and in the
control side of the unilaterally denervated animals, "electrical silence" was
the consistent electromyographic finding at rest in all muscles examined. In
the denervated muscles and in the animals on a vitamin E deficient diet, the
initial finding was "electrical silence" at rest but the picture changed in
time as follows:

In the unilaterally denervated animals, except for some transient
irritation potentials on the first post-operative day, the classical denerva-
tion fibrillation potentials began to appear on the fourth post-operative day
and remained present throughout the course of the thirty days of experimental
procedure.

In the vitamin E deficient animals, transient, "irritation type"
potentials were found after the tenth day. At fifteen to twenty days, persist-
ent, spontaneous, asynchronous, "fibrillation-type" potentials appear and
remained present until the death of the animals.
Comparing the spontaneous potentials of denervation with those seen in vitamin E deficient muscle, no differentiation was observed on the basis of the parameters and character as recorded by the oscilloscope tracing. That is, voltage, duration, frequency of discharge (both are asynchronous) and wave form were essentially identical.

In the vitamin E deficient animals, motor unit potentials appeared normal up to the fifteenth post-diet day. That is, single motor unit potentials produced by the animal have the same parameters as single motor unit potentials of the control or normal muscles.

After eighteen days, total voltage output (as motor unit potentials) was below normal voltage. This observation was based on many EKG "samples" of each muscle and on the production of the motor unit potentials by effort of the animal and reflex stimulation of the muscle.

In the acute stages of the vitamin E deficiency the parameters of voltage and duration appeared markedly reduced. Only potentials with low voltage and short duration could be elicited. Voluntary or reflex contractions of the vitamin E deficient muscles produced bursts of high frequency potentials. It was difficult and for the most part impossible to elicit "single" motor unit potentials by any amount of reflex stimulation, positioning of the limb or prodding of the animal. It was significant, however, that motor unit activity (though of abnormal appearance) could always be elicited up to the time of the animals demise, indicating the existence of some functional neuronal connections to the muscle.
The presence of some functional neuronal connections corresponds well to the electrodiagnostic findings of "early" or "incomplete" denervation in these E deficient animals, seen particularly in the discontinuities of the strength-duration curves. Also consistent with these findings is the presence of fibrillation potentials in the E deficient muscles which are indistinguishable from the fibrillation potentials of the denervated animals.

(2) Comparison of the electromyographic findings to those of previous investigators.

No previous reports have appeared in the literature of electromyo-
graphic studies with vitamin E deficient skeletal muscles. The findings of
fibrillation potentials at rest and the altered motor unit electromyographic
picture represent original findings.

The presence of fibrillation potentials in denervated muscle is
consistent with the findings of earlier investigators.

c. Effect of fasting on electrodiagnosis and electromyography
findings.

Since the changes in electrodiagnosis data of the vitamin E deficient
animals occurred at about the same time as the marked weight loss seen in these
animals (average loss of 21.6% of total body weight in five days), the possi-
bility existed that some of the EON changes might be due to inanition alone.

Electrodiagnosis and electromyography data were collected from a
group of fasting animals not in an E deficient diet program but on a diet which
would produce a comparable weight loss (about 20.0%) during a comparable period
of time (4-6 days).
In this investigation it was found that all electrodiagnosis as well as electromyography findings in these fasted animals were within normal limits. On the basis of these findings, it is concluded that changes in electrodiagnosis as well as electromyography data of vitamin E deficiency are not directly related to weight loss. To illustrate this graphically, weight changes of the E deficient animals and of the fasted animals (positioned on the graph to coincide with the beginning of weight loss of the E deficient animals) were plotted together with chronic of control, E deficient, and fasted animals (Figure 19).

It may be seen that while the weight loss curves parallel each other in the E deficiency and fasted groups, the chronic values did not follow the E deficiency changes but remained at normal or control levels.

2. Pharmacology.

The exact mechanism of neuromuscular transmission, whether electrical, chemical or both, has not been fully established. Notwithstanding this, several drugs are reported to exert their principle effects at the myoneural junction by influencing neuromuscular transmission. These include: d-tubocurarine, decamethonium, succinylcholine, and prostigmine. D-tubocurarine is reported to produce a block in neuromuscular transmission by competitive inhibition, preventing access of acetylcholine to the receptor sites preventing muscle depolarization. On the other hand, decamethonium and succinylcholine affect transmission in a manner similar to an excess of acetylcholine, that is, causing persistent depolarization of the myoneural junction thereby preventing repolarization and repetition of the transmission cycle. This difference in
pharmacologic activity between curariform drugs of the "blocking type" and those of the "depolarization type" is not absolute (Jarcho et al., 1951).

Prostigmine is described as a powerful anti-curare agent. It is reported that this effect is brought about in two ways. First, by an indirect
effect wherein prostigmine inhibits cholinesterase which destroys acetylcholine. There is a consequent accumulation of greater than normal amounts of acetylcholine which can compete with curare for the cholinergic receptor sites. Second, a direct effect is produced on skeletal muscle probably at the myoneural junction. Lehman, 1946, showed that prostigmine caused contracture of the frog's rectus muscle and suggested therefore that this effect was partly due to a direct action of the drug on the myoneural junction. The direct skeletal muscle stimulating action of prostigmine was shown experimentally on cat muscle by Riker and Wescoe, 1946. There is documentation, therefore, that prostigmine activity is not limited to an anti-cholinesterase effect alone, but a direct muscle stimulating effect may also be produced.

a. Results of the present investigation.

In the present investigation, intravenous administration of d-tubocurarine with continuous electromyographic recording showed the following effects: In doses slightly less than full neuromuscular blocking doses, (100 ug/kg) no effect was to be seen on fibrillatory activity in the E deficient and chronically deservated muscles. In full blocking doses (200 ug/kg) with the animals maintained on artificial respiration no change in activity of the fibrillating muscles was observed. With this dose no motor response was elicited by direct electrical stimulation of the nerve to the vitamin E deficient muscle and the nerve to the opposite normally innervated muscle in unilaterally deservated animals. This situation obtained in all
seven E deficient and seven denervated animals tested. In several denervated 
and also in several E deficient animals, the blocking dose of d-tubocurarine 
was doubled but no apparent effects on fibrillation were seen. It is signif-
icant in this study that comparable doses of d-tubocurarine whether less than 
full blocking dose, full blocking dose, or double the full blocking dose had 
no effect on either the fibrillation potentials of denervated muscles or 
those of vitamin E deficient muscles. That is to say, both types of fibrilla-
tion reacted similarly in this experimental situation.

Intravenous administration of succinyl choline (200 mg/kg) in the 
normal animal produced a "shower of activity" which has been described as a 
"wave of depolarization". This was seen in these animals for about two 
minutes, following which activity ceased and electrical silence was the 
electromyographic finding. Motor unit activity associated with effort began 
to return to the anterior tibial muscles examined after ten to fifteen 
minutes. This neuromuscular block was obtained without causing respiratory 
arrest. In the vitamin E deficient animals as well as in chronically 
denervated animals, a characteristic "shower of activity" followed intravenous 
administration of small doses of succinyl choline. In both groups of animals, 
atal activity ceased after about two minutes. This includes the motor unit 
action potentials of the vitamin E deficient animals and the spontaneous 
potentials of both the E deficient and denervated animals. The spontaneous 
potentials of the vitamin E deficient animals were observed to return after 
ten minutes and always preceded the return of motor unit activity. Fibrilla-
tion potentials of the denervated animals were also seen to return after about
ten minutes. These changes as recorded with the electromyograph, are illustrated in Figure 17.

Events identical to those described above for succinyl choline, were observed following decamethonium. Decamethonium in small doses was administered to three each of denervated and vitamin E deficient animals. However, whereas activity following succinyl choline returned after ten minutes, activity following decamethonium did not return for at least twenty minutes.

In summary then, no difference in effect was observed between the response of fibrillation of denervation and that of vitamin E deficient muscle to either succinyl choline or decamethonium.

After administration of prostigmine the following effects were noted: In normal animals with innervated muscles, administration of prostigmine resulted in augmentation of the motor unit potentials. Minimal effort by the animal resulted in prolonged bursts of motor unit activity, usually with an increase in amplitude. No spontaneous potentials were elicited from these muscles at rest. In the vitamin E deficient animals, five minutes after the administration of prostigmine, there was an augmentation of motor unit activity such as seen in the normal rabbits. The increase in motor unit activity had the effect of "masking" any changes which may be produced in the spontaneous potentials. When the sciatic nerve to the recording muscle was severed, and prostigmine given, the motor unit activity was absent. It was then possible to detect the increase in amplitude and frequency of discharge of the spontaneous potentials, which had occurred. In the chronically
denervated animals, the administration of prostigmine resulted in an increase
in amplitude or voltage and in the frequency of the fibrillation potentials.
The effect of prostigmine on the electromyographic recordings of the
potentials of normal, vitamin E deficient and chronically denervated animals
are illustrated in Figure 13.

In the use of prostigmine, the effects observed on both types of
fibrillation, those from denervation and those from vitamin E deficient muscle,
were the same.

In summary, then, d-tubocurarine, decamethonium, succinyl-choline,
and prostigmine, had a similar effect on the fibrillation potentials of
denervated rabbits and the fibrillation potentials of vitamin E deficient
rabbits. This invariable correlation of response with all four drugs is
strong evidence for the statement that these two types of fibrillation are
identical.

b. Comparison of the pharmacological findings with those of
previous investigators.

None of the drug effects on fibrillation potentials of vitamin E
deficient skeletal muscles have been previously reported.

In the effect of d-tubocurarine on denervation fibrillation, our
findings are in agreement with those investigators who report no effect on
fibrillation when this drug is administered in doses causing "muscle paralysis"
as determined by electrical stimulation of a contralateral normal limb
(Langley and Kato, 1914-15; Solanet and Magladery, 1940; Eccles, 1941; Reid,
1942). McIntyre (1945) working with dogs found that while "blocking doses" of \( \text{d-tubocurarine} \) did not affect fibrillation, very large doses, administered by close intra-arterial injection, did cause cessation of fibrillation activity. Jarcho et al (1950, 1951) found that this same situation obtains in the rat. They were able to cause a slight increase in activity prior to cessation of activity and suggest that \( \text{d-tubocurarine} \) is a weak "depolarizer" which is able to bring about sustained depolarization of the myoneural junction or muscle fiber leading to cessation of fibrillatory activity. The inability in this investigation to produce this effect may be due to: 1) the fact that much larger doses of \( \text{d-tubocurarine} \) may be required to reproduce their effects in the rabbit; or 2) a species difference, for Jarcho and his co-workers employed the rat and the present investigation used the rabbit. This point may be settled by further work with much larger doses of \( \text{d-tubocurarine} \) using denervated muscles in rabbits.

The "depolarization" effect of decamethonium and succinylcholine on denervated muscle with cessation of fibrillation, reported by Jarcho et al (1950), Laimis (1951) and Riker (1957), is confirmed by this investigation. The finding in this investigation that prostigmine brings about increased fibrillation activity in denervated muscle is in agreement with the reports of Rosenbluth and Luco (1937), Riker and Wescoe (1946) and Riker et al (1957). Riker suggests that prostigmine has a direct effect on muscle possible acting as a depolarizing agent. If this be true, then it should follow that in large doses, a "sustained" depolarization may be produced and should cause cessation of fibrillation activity as seen with decamethonium.
and succinylcholine.

B. Findings presented but not validated by this experiment.

1. Site of origin of fibrillation potentials.

To test the premise that the fibrillation potentials seen in vitamin E deficient skeletal muscle, like the fibrillation potentials of denervated skeletal muscle, are independent of central nervous system control, the following experiment was performed. The motor nerves to fibrillating anterior tibial muscles of vitamin E deficient animals were severed while the animals were unanesthetized. In each of the seven animals upon which this procedure was carried out, all motor unit activity ceased but the fibrillation potentials continued to fire. On the basis of this finding it was concluded that fibrillation of E deficient muscle like fibrillation of denervated muscle is independent of central nervous system control, its site of origin probably residing either in the motor end plate or in the muscle fiber itself.

In view of the above finding, the status of the motor end plate and the muscle fiber in these conditions, vitamin E deficiency and denervation, assume special significance in attempting to ascertain the site of origin of the fibrillation potentials seen in these two myopathies. The following summary presents what appears to be the current knowledge of the histological status of the motor end-plate and muscle fiber in normal, denervated, and vitamin E deficient muscles. A review of the literature dealing with investigations which have been carried out on the site of origin of fibrillation potentials in denervated muscles is also presented.
Considering the normal structure of the motor end plate, Reger (1955) presents the following view (which is in agreement with the work of Couteaux 1947):

The axon (a), along with myelin (M) and sheath components (endo-neurium and neurilemma), of which the nuclei (E) and (N) are indicated, approaches the muscle fiber. The nerve sheath elements have continuity with the muscle fiber sheath components and the myelin terminates before the axon arborizes. The arborizing axon has associated with it nuclei ('arborization' nuclei, AN). The terminal axonal branches are situated in depressed grooved regions of the sarcolemma. These sarcolemmal grooved areas represent the post-synaptic membrane and are differentiated to the extent that they have invaginated from their surface projections directed into the sole-plasma, thus greatly increasing surface area. In the sarcoplasma of the end-plate region (sole-plate), muscle nuclei ('fundamental' nuclei, FN) and mitochondria are found. The Z membranes (Z), are shown as extending for some distance into the sole-plate."

These components are diagrammatically shown in Reger's figure reproduced here as Figure 29.

In denervation due to nerve section, only the axon and its arborization within the motor end plate degenerates (Wallerian degeneration). This is reported as occurring as early as two days following motor nerve section (Gutmann and Young, 1944; Adams, Denny-Brown and Pemyacker, 1951). The remaining elements of the motor end plate remain in contact with the muscle fiber for a considerable period (up to 17 months) "with relatively little change". "The change in muscle fibers due to denervation is one of atrophy and consists in a reduction of size of the muscle fibers while their form is essentially conserved. Gutmann and Young (1945) point out that "when a muscle fibre shrinks its end-plate may remain intact, though shrunken, even after more than a year of denervation."
Fig. 1 A diagram of a semi-profile view of a motor end-plate and its associated nerve and muscle fiber.

A, axon
AN, "arborization" nucleus
E, nucleus of Henle sheath (endoneurium)
FN, "fundamental" nucleus
M, myelin
MN, muscle fiber nucleus
N, neurilemma nucleus
SL, sarcolemma
Z, Z membrane of muscle fiber

FIGURE 20
A DIAGRAM OF A SEMI-PROFILE VIEW OF A MOTOR END-PLATE AND ITS ASSOCIATED NERVE AND MUSCLE FIBER (REGER, 1955)

In vitamin E deficiency the pathology is one of degeneration of the muscle fiber, only, with a destruction of cells or tissue and a change in appearance to such a degree that recognition of the muscle fiber is difficult or no longer possible (Figures 6 and 7). The neurites and nerve end plates remain intact thought they become anatomically or histologically detached.
from the muscle fiber. This has been confirmed by Rogers, Pappenheimer and Goettch (1931) in guinea pigs and rabbits; by Pappenheimer (1939) for rats; and for rabbits, by Oatrz and Houchin (1951) and more recently by Anderson and Rickard (1956). The essential picture then is: in denervation the nerve fibers degenerate leaving the motor end plate in contact with the muscle fiber which itself atrophies; and in vitamin E deficiency the nerve fibers and the nerve end plates remain intact, the motor end plates become detached from the muscle fiber which is undergoing degeneration.

The histologic changes in the motor end plate in denervation in vitamin E deficiency are shown in Figure 21. In this picture, A taken from Oltmann and Young (1945) shows the normal end plate with intact axon. In B, also from Oltmann and Young, denervation has resulted in the degeneration of the axon leaving the motor end plate attached to the muscle fiber. In C, taken from Pappenheimer (1939) the intact axon is shown attached to the motor end plate but connection with a muscle fiber has been lost.

The status of the nerve end plate assumes importance when considered in terms of experimental work done on the site of origin of fibrillation potentials. In 1941, Eccles reported that he had investigated the fibrillatory movements of denervated cat muscle due to contraction set up by impulses arising spontaneously from single muscle fibers. Using fine electrodes for leading from small bundles of muscle fibers, he found that in a single fiber the rhythm of discharge was about 10 per second. By taking electrical recordings at different points along the length of the fibers, he showed that
this rhythmic discharge is set up at a focus often widely separated from the region of the motor nerve endings on the muscle fiber, and from this focus, the impulses are propagated normally along the length of the whole fiber. This information was presented in the form of a lecture with no other details nor mention of histological corroboration of the location of the end plates.

Hayes and Woolsey (1942) undertook to study the problem of whether the unit of fibrillar activity in denervated striated muscle is the entire muscle fiber or a fraction thereof. Using unilaterally denervated diaphragm of the rat, they found that the potential changes do not originate at random throughout the muscle as would be expected if fibrillation were limited to parts of muscle fibers. Rather, fibrillation potentials were found to take origin from a very specific place -- the end-plate zone, at the very center of the muscle fibers -- and spread thence toward both ends. In spite of asynchronous activity in various units, they report, it is easy to demonstrate that the contractions originate at the end plate zone even in vigorously fibrillating muscle. Further evidence for the site of origin of the fibrillar contraction was secured by excising the end-plate zone from actively fibrillating muscle. Fibrillation continued in the strip of muscle bearing the end-plate zone but ceased in the other sections as soon as they were cut off.

Jarcho et al (1954) reported on their experimental work on the site of origin of fibrillar potentials in denervated skeletal muscle. Their findings are that fibrillar potentials originate within or in the immediate
FIGURE 21

PHOTOMICROGRAPHS SHOWING STATUS OF NERVE AND MOTOR END PLATE IN NORMAL, DENERVATED AND VITAMIN E DEFICIENT MUSCLES

A -- Normal motor end plate with intact axon (Gutmann and Young, 1945).

B -- Motor end plate in denervation. (Gutmann and Young, 1945).

C -- Motor end plate and axon in vitamin E deficiency. (Pappenheimer, 1939).
neighborhood of the denervated end-plates, at least in the anterior gracilis muscle of the rat. With careful placement of the electrodes, they state, it was possible always to outline the end-plate zones quite sharply by watching for points at which the phase order of fibrillar potentials became reversed. In all cases, there was accurate agreement with previous histological and physiological evidence of the location of the end-plates. In a previous paper, Jarcho and his co-workers (1952) reported mapping the location of end-plate "zones" by locating points at which end-plate potentials could be elicited in the normal anterior gracilis muscle of rats. They found that the end-plates are to be found in two restricted zones. Further, study of action potentials elicited by nerve stimulation showed that there was the occasional occurrence of doubly innervated muscle fibers. Histological preparations confirmed the location of the end-plate zones and the existence of the doubly innervated fibers. In their 1954 paper on fibrillation sites, they found that fibrillation potentials emanated from both end plate zones, and that these potentials could be followed from the end-plate zones out to both tendons.

On the basis of the findings presented in this study, it is not possible to conclude which situation obtains: 1) fibrillation is associated only with the presence of the motor end-plate on the muscle fiber, or 2) muscle fibers may fibrillate in the absence of the motor end-plate. The work of Hayes and Woolsey (1942) and that of Jarcho et al (1952, 1954) supports the former view; the histological data on the status of the motor end plate
in E deficiency, and the work of Eccles (1941) on denervated muscle favors the latter view. Additional research on the problem of the origin of fibrillation in vitamin E deficient skeletal muscle is necessary. Possible approaches to an investigation of this type are described in "D.-Future Problems".

C. Contribution of this investigation to existing science.

This work represents the first definitive study of the skeletal muscle pathology of vitamin E deficiency using electrodiagnostic (rhoebase, strength-duration, chronaxie, repetitive stimulation and galvanic tetanus ratio), electromyographic and pharmacologic (using neuro-muscular drugs) techniques.

On the basis of the investigative findings, disruption of the motor units and denervation of some of the muscle fibers in affected muscles of vitamin E deficient rabbits is clearly established.

Among the electrophysiological findings the most significant appears to be that of the presence of spontaneous fibrillation potentials seen electromyographically in these dystrophic animals. This significance is emphasised when it is considered that fibrillation potentials in skeletal muscle have been reported as only occurring when section and subsequent degeneration of the motor nerve to the muscle has taken place as with surgical or traumatic denervation. In vitamin E deficiency the primary disruption is in the muscle fiber itself with no reported degeneration of the motor nerve
by all previous investigators.

The experimental pharmacology with neuromuscular drugs undertaken in this investigation represents the first study of such agents as to their effect on the fibrillation potentials of vitamin E deficient muscle as well as the first contrasting of such data to the effect of the same drugs on fibrillation potentials of denervated muscle.

The necessity for comparing denervation data to E deficiency data required that all investigative procedure used on E deficient muscle also be used on denervated muscle. Thus, a re-evaluation has been made of electrodiagnostic and electromyographic findings in experimental denervation. Specific contributions have been made to newer EDX techniques (repetitive stimulation) and to controversial EDX techniques (rhoebase, galvanic tetanus ratio and faradic stimulation).

A method is presented for the handling of electrodiagnosis data (particularly that of strength-duration curves) which has been previously considered as too variable to lend itself to statistical analysis.

D. Future problems.

In most investigations of a research nature, many more new problems are posed than may be solved by the current investigation itself. This research endeavor proves to be no exception to this statement. Among the problems touched upon in the present work and which should be investigated in the future, the following may be briefly cited.
The pharmacological findings in this investigation suggest that additional investigation be carried out with large doses of d-tubocurarine and procaine with special emphasis placed on the reported "depolarizing" effects of these drugs on fibrillation potentials of denervated muscle.

Reversal of nutritional muscular dystrophy effects with alpha-tocopherol should be studied using the electrodiagnosis and electromyography procedures employed in this investigation. This would permit an evaluation to be made of these procedures for use as bioassay tools in nutritional muscular dystrophy research. Future use of these techniques could be made in evaluating drugs other than tocopherol which are capable of reversing the muscle pathology in vitamin E deficiency.

Electromyographic techniques using microelectrodes should be applied to isolated fibrillating muscle fibers of vitamin E deficient muscle to determine whether the locus of excitation leading to the production of fibrillation potentials is restricted to a particular portion of the muscle fiber or whether many loci of excitation may be present in the individual fiber.

Finally, the importance of the status of the myoneural junction in vitamin E deficiency, pointed up by this investigation, suggests that electron microscopy studies be carried out on this portion of the degenerating muscle fiber to determine the condition of the nervous and muscle elements which make up the "motor end plate".
CHAPTER V

SUMMARY

Vitamin E deficiency was produced in rabbits maintained on a low vitamin E diet. Electrodiagnosis and electromyographic data were collected from the anterior tibial muscles of these animals and contrasted against a background of comparable data obtained from:

1. Control animals on the same diet with vitamin E (as alpha tocopherol).
2. Denervated animals maintained on the control diet.
3. Acutely fasted animals on a low caloric diet but one which contained the vitamins (including E) and minerals found in the control diet.

Electrodiagnosis revealed the following:

1. Strength-duration curves of vitamin E deficient skeletal muscles in the acute deficiency stage had the same upward trend as completely denervated muscles, indicating a change in excitability. The E deficient muscle curves were complex, containing two segments with a discontinuity or hink at their juncture. This finding suggests an incomplete or partial denervation for it is characteristically found in re-innervated muscle following complete denervation due to sectioned nerve, and in partly denervated muscles due to partially injured motor nerves and in crushed nerve injuries. Strength-duration curves of the fasted animals were normal.
2. Chronaxie, repetitive stimulation and galvanic tetanus ratio values of vitamin E deficient muscle in the late stages were found to be comparable to the findings in the early stages of completely denervated muscle. Fasted animals had normal values for all procedures.

3. Percutaneous faradic stimulation results proved to be unusual in that the response of completely denervated muscle persisted throughout the entire investigation time of sixty days. Autopsy examination on these animals confirmed the continued severed state of the motor nerve. This is contrary to earlier reports that response of completely denervated muscle to percutaneous faradic stimulation is lost (in experimental animals and in humans) after fifteen days. Some decrease in threshold of the denervated muscles was found. No change in threshold for the vitamin E deficient animal muscles as well as the fasted animal muscles was obtained.

A method is presented for converting electrodagnosis data to "threshold ratios" which permits an efficient means for comparing data directly and by statistical evaluation.

Electromyographic studies revealed:

1. In muscles at rest, the vitamin E deficient muscles produced spontaneous fibrillation-like potentials whose parameters (voltage, duration, and frequency) were indistinguishable from those of the classical fibrillation potentials of denervation.

2. Motor unit potentials of vitamin E deficient muscles were observed to change as the deficiency progressed, showing: decrease in voltage and
duration and loss of ability to recruit complete motor units. The resemblance of these potentials to "dystrophic potentials" seen in humans is discussed.

3. Studies on the effect of drugs on the spontaneous potentials of E deficiency and denervation revealed the following:

a. d-Tubocurarine, in full blocking and in twice the blocking dose had no effect on either of these two types of fibrillation potentials.

b. Succinyl choline and decamethonium in small doses (a fraction of the blocking dose) brought about an immediate increase in potentials followed by cessation of all electrical activity in the denervated as well as in the E deficient muscles.

c. Prostigmine brought about an increase in voltage and frequency of discharge of the spontaneous potentials seen in denervated and in vitamin E deficient muscles.

Considering the regular occurrence of similarity between the electromyography and pharmacology data obtained from vitamin E deficient and from denervated muscles, it is concluded that the spontaneous potentials which occur in each of these conditions, so-called fibrillation potentials, are indistinguishable from each other. This striking similarity between these fibrillation potentials is all the more unusual for two reasons: (1) on the basis of the electrodagnosis and electromyography evidence presented in this investigation, there is a "partial denervation" which occurs in vitamin E deficient skeletal muscle, and (2) the process by which this "denervation" is accomplished appears to be due to two different mechanisms. In the case of
vitamin E deficiency, "denervation" is apparently due to a metabolic break-
down in the skeletal muscle leading to degeneration of the muscle fibers
with some alteration in the myoneural junction, while in surgically denervated
skeletal muscle the principal alteration consists of a degeneration of the
motor nerve fiber with consequent alteration in the myoneural junction.
BIBLIOGRAPHY


APPENDIX I

STATISTICAL EVALUATION OF "RAW" STRENGTH-DURATION CURVE DATA AND THESE DATA CONVERTED TO THRESHOLD-RATIO VALUES

A. Introduction.

Strength-duration curves express the relationship between the time during which a stimulus is applied and the strength of that stimulus required to produce a threshold response. Certain factors affect the threshold or level of the strength-duration curve. The most important of these are skin temperature, blood supply, edema, and the electrode position. In 1952, Harris (Ann. Phys. Med., 1:126, 1952) made a study of these factors as they relate to changes in threshold. He found that an increase in temperature lowers threshold or rheobase while a decrease in temperature increases threshold. A high threshold curve is obtained in the presence of edema. Ischemia causes a rise in threshold of the curve. Position of the stimulating electrode in relation to the motor point will also affect threshold -- rheobase increases as the distance of the electrode from the motor point increases.

For data to be useful in experimental procedures, they should lend themselves to comparison by inspection as well as by statistical evaluation. To standardize the strength-duration data obtained in the experimental investigation of vitamin E deficiency effects on skeletal muscle so as to allow
comparison of the data obtained, all points on a strength-duration curve were
made a function of the rheobase for that particular curve. That is, all
threshold values for the different durations of the stimuli for a given curve
were divided by the rheobase for that curve. Rheobase now becomes unity and
all other points on the curve are unity or greater.

In order that we get some idea of the standard error of measurement
in the process of measuring a strength-duration curve, two groups of data have
been collected. The first group, Table XXII, represents three curves made upon
one animal (S 244) in prompt succession of each other so as to minimize the
influence of temperature, ischemia, and edema (that is, these factors would
remain essentially unchanged). Variability in rheobase was introduced by
deliberately shifting the electrode slightly after the data for each curve were
recorded. The second group, Table XXIV, represents four curves compiled from
the same animal (C 260) on four separate days. All factors which may affect
the rheobase would likely be represented in this second group: temperature,
ischemia, edema, and electrode position.

All the data of these two groups were converted to threshold ratios
and are presented in Tables XXIII and XXV respectively.

One set of apparatus and one operator were employed in making all of
the observations.

B. Statistical Evaluation of the Data.

An analysis of the variances between strength-duration curve data as
"raw" data and following conversion to threshold ratio values was carried out
for two experimental conditions, namely, three curves on the same animal in
prompt succession, and four curves on another animal on different days.
<table>
<thead>
<tr>
<th>Duration of Stimulus (Milliseconds)</th>
<th>First Curve</th>
<th>Second Curve</th>
<th>Third Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2.58</td>
<td>3.10</td>
<td>1.98</td>
</tr>
<tr>
<td>0.3</td>
<td>1.72</td>
<td>1.92</td>
<td>1.25</td>
</tr>
<tr>
<td>0.6</td>
<td>1.28</td>
<td>1.58</td>
<td>1.01</td>
</tr>
<tr>
<td>1.0</td>
<td>1.03</td>
<td>1.45</td>
<td>0.91</td>
</tr>
<tr>
<td>3.0</td>
<td>0.86</td>
<td>1.22</td>
<td>0.77</td>
</tr>
<tr>
<td>6.0</td>
<td>0.77</td>
<td>1.02</td>
<td>0.64</td>
</tr>
<tr>
<td>10</td>
<td>0.79</td>
<td>0.94</td>
<td>0.60</td>
</tr>
<tr>
<td>30</td>
<td>0.68</td>
<td>0.92</td>
<td>0.59</td>
</tr>
<tr>
<td>60</td>
<td>0.62</td>
<td>0.80</td>
<td>0.52</td>
</tr>
<tr>
<td>100</td>
<td>0.60</td>
<td>0.78</td>
<td>0.50</td>
</tr>
<tr>
<td>300</td>
<td>0.60</td>
<td>0.78</td>
<td>0.50</td>
</tr>
</tbody>
</table>
TABLE XXIII

STRENGTH-DURATION DATA (AS THRESHOLD RATIOS) FROM THREE SUCCESSIVE CURVES MADE ON THE SAME ANIMAL (E 244) FOLLOWING REPOSITIONING OF THE ELECTRODE.

<table>
<thead>
<tr>
<th>Duration of Stimulus (Milliseconds)</th>
<th>First Curve</th>
<th>Second Curve</th>
<th>Third Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>.1</td>
<td>4.29</td>
<td>3.97</td>
<td>3.98</td>
</tr>
<tr>
<td>.3</td>
<td>2.57</td>
<td>2.46</td>
<td>2.50</td>
</tr>
<tr>
<td>.6</td>
<td>2.14</td>
<td>2.03</td>
<td>2.02</td>
</tr>
<tr>
<td>1.0</td>
<td>1.71</td>
<td>1.86</td>
<td>1.82</td>
</tr>
<tr>
<td>3.0</td>
<td>1.43</td>
<td>1.56</td>
<td>1.53</td>
</tr>
<tr>
<td>6.0</td>
<td>1.29</td>
<td>1.31</td>
<td>1.29</td>
</tr>
<tr>
<td>10</td>
<td>1.17</td>
<td>1.21</td>
<td>1.20</td>
</tr>
<tr>
<td>30</td>
<td>1.14</td>
<td>1.18</td>
<td>1.18</td>
</tr>
<tr>
<td>60</td>
<td>1.03</td>
<td>1.03</td>
<td>1.03</td>
</tr>
<tr>
<td>100</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>300</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>
# Table XXIV

**Strength-Duration Data (in Milliamperes) as Four Curves Collected from the Same Animal (C 260) on Four Different Days.**

<table>
<thead>
<tr>
<th>Duration of Stimulus (Milliseconds)</th>
<th>First Day</th>
<th>Seventh Day</th>
<th>Sixteenth Day</th>
<th>Twenty-sixth Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>.1</td>
<td>3.96</td>
<td>2.25</td>
<td>2.73</td>
<td>4.80</td>
</tr>
<tr>
<td>.3</td>
<td>2.27</td>
<td>1.13</td>
<td>1.38</td>
<td>2.50</td>
</tr>
<tr>
<td>.6</td>
<td>1.46</td>
<td>0.85</td>
<td>1.02</td>
<td>1.75</td>
</tr>
<tr>
<td>1.0</td>
<td>1.20</td>
<td>0.70</td>
<td>0.85</td>
<td>1.46</td>
</tr>
<tr>
<td>3.0</td>
<td>0.94</td>
<td>0.57</td>
<td>0.68</td>
<td>1.15</td>
</tr>
<tr>
<td>6.0</td>
<td>0.82</td>
<td>0.50</td>
<td>0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>0.80</td>
<td>0.50</td>
<td>0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>30</td>
<td>0.80</td>
<td>0.50</td>
<td>0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>60</td>
<td>0.80</td>
<td>0.50</td>
<td>0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>100</td>
<td>0.80</td>
<td>0.50</td>
<td>0.60</td>
<td>1.00</td>
</tr>
<tr>
<td>300</td>
<td>0.80</td>
<td>0.50</td>
<td>0.60</td>
<td>1.00</td>
</tr>
</tbody>
</table>
TABLE XXV

STRENGTH-DURATION DATA CONVERTED TO "THRESHOLD RATIOS"
FROM FOUR CURVES COLLECTED ON THE SAME ANIMAL
(C 260) ON FOUR DIFFERENT DAYS.

<table>
<thead>
<tr>
<th>Duration of Stimulus (Milliseconds)</th>
<th>First Day</th>
<th>Seventh Day</th>
<th>Sixteenth Day</th>
<th>Twenty-sixth Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>.1</td>
<td>4.95</td>
<td>4.50</td>
<td>4.55</td>
<td>4.80</td>
</tr>
<tr>
<td>.3</td>
<td>2.84</td>
<td>2.26</td>
<td>2.30</td>
<td>2.50</td>
</tr>
<tr>
<td>.6</td>
<td>1.83</td>
<td>1.69</td>
<td>1.70</td>
<td>1.75</td>
</tr>
<tr>
<td>1.0</td>
<td>1.50</td>
<td>1.40</td>
<td>1.42</td>
<td>1.46</td>
</tr>
<tr>
<td>3.0</td>
<td>1.17</td>
<td>1.13</td>
<td>1.13</td>
<td>1.15</td>
</tr>
<tr>
<td>6.0</td>
<td>1.02</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>30</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>60</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>100</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>300</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>
The total variance in each set of data was separated into three parts: (1) variance between durations of stimuli, (2) variance between duplicates or curves, and (3) variance due to experimental error.

Following Snedecor (Statistical Methods Applied to Experiments in Agriculture and Biology, 1946) the variance ratio, $F$, was obtained by taking the ratio of variance between durations, variance due to experimental error, and the ratio of variance between duplicates or curves/variance due to experimental error. The $F$ values thus obtained were compared with a table of critical $F$ values judged at stated probability levels (in this case the 1% or the .01 level).

Summaries of the analysis of variance for all the data are presented in Tables XXVI, XXVII, XXVIII, and XXIX.

1. Variance of the three successive curves.

An examination of Table XXVI, analysis of "raw data, shows that the variance ratio, $F$, between durations and between duplicates is highly significant when judged at either the .05 or the .01 level of probability. When these data were converted to threshold ratios and analyzed, Table XXVII, the variance ratio of "between durations" became larger and therefore much more significant than the "between durations" of the raw data. On the other hand, the variance ratio of "between duplicate curves" of threshold ratio data was found to decrease, and was not significant at any level of probability.

A statistically significant difference exists between strength-durations curves as raw data obtained at the time of electrodiagnosis.
### TABLE XXVI

**Analysis of the Variance Between Three Successive Strength-Duration Curves (as Raw Data)**
Made on the Same Animal (E 244) Following Repositioning of the Electrode.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>Variance Ratio</th>
<th>Level of Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between durations</td>
<td>10.3208</td>
<td>10</td>
<td>1.0321</td>
<td>58.3107</td>
<td>3.37 2.35</td>
</tr>
<tr>
<td>Between duplicate curves</td>
<td>1.2604</td>
<td>2</td>
<td>.6302</td>
<td>35.6045</td>
<td>5.85 3.49</td>
</tr>
<tr>
<td>Experimental error</td>
<td>.3559</td>
<td>20</td>
<td>.0177</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Totals</td>
<td>11.9371</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### TABLE XXVII

**Analysis of the Variance Between Three Successive Strength-Duration Curves (as Threshold Ratios)**
Made on the Same Animal (E 244) Following Repositioning of the Electrode.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>Variance Ratio</th>
<th>Level of Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between durations</td>
<td>25.8950</td>
<td>10</td>
<td>2.5895</td>
<td>507.7450</td>
<td>3.37 2.35</td>
</tr>
<tr>
<td>Between duplicate curves</td>
<td>.0023</td>
<td>2</td>
<td>.0011</td>
<td>.2156</td>
<td>5.85 3.49</td>
</tr>
<tr>
<td>Experimental error and interaction</td>
<td>.1025</td>
<td>20</td>
<td>.0051</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Totals</td>
<td>25.9998</td>
<td>32</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### TABLE XXVIII

Analysis of the variance between four strength-duration curves (as raw data) made on one animal (c 260) on four different days.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>Variance Ratio</th>
<th>Level of Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between durations</td>
<td>27.4961</td>
<td>10</td>
<td>2.7486</td>
<td>31.7390</td>
<td>2.98 2.16</td>
</tr>
<tr>
<td>Between curves</td>
<td>4.7254</td>
<td>3</td>
<td>1.5751</td>
<td>18.1882</td>
<td>4.51 2.92</td>
</tr>
<tr>
<td>Experimental error</td>
<td>2.6006</td>
<td>30</td>
<td>.0866</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Totals</td>
<td>34.8121</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### TABLE XXIX

Analysis of the variance between four strength-duration curves (as threshold ratios) made on one animal (c 260) on four different days.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>Variance Ratio</th>
<th>Level of Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between durations</td>
<td>51.1213</td>
<td>10</td>
<td>5.1121</td>
<td>580.9204</td>
<td>2.98 2.16</td>
</tr>
<tr>
<td>Between curves</td>
<td>.1011</td>
<td>3</td>
<td>.0337</td>
<td>3.8295</td>
<td>4.51 2.92</td>
</tr>
<tr>
<td>Experimental error</td>
<td>.2642</td>
<td>30</td>
<td>.0088</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Totals</td>
<td>51.4866</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
these strength-duration data are converted to threshold ratios, no statistical significance can be found between the curves and we may say that they are identical. Further, it may be concluded that when all factors which affect skin resistance are kept nearly identical, difference in position of the electrode, which alone can account for changes in threshold, has no effect on the strength-duration curve itself.

2. Variance of the four curves made on different days.

In Table XXXIII, the analysis of the variance of "raw" data, a highly significant difference is found between durations and between curves when judged at either level of probability, .05 or .01. Analysis of these raw data converted to threshold ratios, Table XXXIV, shows that the variance ratio between durations is made much more significant.

The variance ratio between curves is not significant when judged at the .01 level of probability, the level at which the data is to be judged in this procedure. It is interesting to note that when the variance ratio between curves is judged at the .05 level, there is a difference between the curves. This is explained on the basis of the time difference between the collection of the data allowing possible influence of all factors which may affect skin resistance (temperature, ischecemia, edema) as well as the position of the stimulating electrode on these different occasions.

C. Summary and Conclusions.

1. Strength-duration data were collected under two different experimental conditions so as to obtain variable threshold levels:
a. Three curves on the same animal in prompt succession of each other with slight repositioning of the electrode between the data collection for each curve.

b. Four curves collected from another animal on four different days.

2. An analysis was made of the variances between the strength-duration curve as "raw" data and following conversion of this data to threshold ratios. It was found that:

a. As raw strength-duration data, a statistical significance exists between the curves due to the different threshold levels of these curves following conversion of the strength-duration data to threshold ratios, no statistical difference can be found between the curves when judged at the 1% probability level.

b. Following conversion to threshold ratios, the difference "between durations" already significant as raw data, is made much more significant.

These findings obtain for both experimental conditions, the three successive curves as well as the four different day curves.

3. On the basis of the findings represented here, it is concluded that conversion of strength-duration data to threshold ratios is a valid procedure which renders the data more useful for comparison by direct observation as well as by statistical evaluation.