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The Influence of Quinine, Cortisone, d-tubocurarine, and Prostigmine on the Responses of Mammalian Striated Muscle

John Faust Polli

Loyola University Chicago

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THE INFLUENCE OF QUININE, CORTISONE,

d-TUBOCURARINE, and PROSTIGMINE

ON THE RESPONSES OF MAMMALIAN

STRIATED MUSCLE.

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John Faust Polli

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

January
1953
LIFE

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CHAPTER I

INTRODUCTION

There are two general techniques by which the action of a drug on the nerve-muscle preparation may be studied. One is the isolated nerve-muscle preparation wherein the muscle and its motor nerve are removed from the animal and placed in a suitable environment. This technique is usually applied to preparations from cold-blooded animals, frogs, etc., but may be used in certain preparations from warm-blooded animals such as rat phrenic nerve-diaphragm muscle preparations, Bulbring (1946). The second method is the in-situ technique in which the pharmacodynamic action of a drug on the nerve-muscle preparation may be studied. In this procedure the animal is usually narcotized, and the tendon end of a muscle (gastrocnemius, tibialis, etc.) may be attached to an isometric or isotonic lever. The variation in muscle tension on stimulation by electrical shocks to its motor nerve is often recorded on a smoked kymograph drum.

The action of a drug which appears very definite

1
in the isolated preparation will often be modified when introduced into the intact animal. Many reasons for this variation in the action of a drug are known; detoxication, excretion, fixation of the drug, etc. However, when a portion of the intact animal is removed and tested in-vitro, many of these variables no longer operate.

An example of the effect of such a variable which is operative in the intact, but not operative in the isolated preparation, is the alteration in the fatigability of the phrenic nerve-diaphragm muscle preparation following adrenalectomy in the rat, Ramey et al (1950). In the adrenalectomized rat the diaphragm stimulated via the phrenic nerve fatigues rapidly; however, when a portion of the diaphragm and its nerve are isolated and suspended in a suitable medium, it responds to the same stimulation without fatiguing appreciably.

The contraction of a muscle in-situ in response to a stimulus transmitted through its motor nerve, is affected by many drugs. A number of these, such as eserine, prostigmine, di-isopropyl fluorophosphate (DFP), etc., have anticholinesterase activity. Some others which have a curare-like action apparently compete with
acetylcholine for reaction sites on the receptor mechanism of the myoneural junction. The mechanism of action by which agents such as parasympathomimetic drugs potentiate neuromuscular transmission in the normal and abnormal animal is not completely understood.

The fact that living cells shrink in concentrated solutions and swell in dilute solutions is regarded as evidence that the cell is surrounded by a semi-permeable membrane, often called the plasma membrane. In nervous tissue and in muscle this living membrane is electrically polarized with respect to the cytoplasm of the cell.

In recent years numerous attempts have been made to determine the difference in electrical potential between the inside and outside of living cells, especially by Hodgkin and Huxley (1945) and by Ling and Gerard (1949). Using the Ling microelectrode a potential difference of up to 0.1 volt has been measured across the resting membrane of muscle fibers. This resting potential or polarization of the plasma membrane has been attributed by many authors to the difference in ionic concentrations, or to the presence of different ions, on the two sides of the muscle membrane.
In general, extracellular body fluids are poor in potassium and cellular protoplasm is poor in sodium. This dynamic ion concentrating property of living cells is explained at present on the basis of hypothetical exchange-transport systems, and much data in support of such hypothesis has been accumulated.

Fenn and Cobb (1936) showed that following muscle activity potassium is lost from muscle fibers while at the same time sodium is gained. During rest recovery of the original ionic concentrations in the muscle fiber is effected.

In order for this transfer of ions to produce an action potential their exchange at the membrane must be separated in time. If the Na and K ions were simultaneously transferred in equivalent amounts no potential difference would result. The manner of ion transfer has been explained by assuming that sodium enters the muscle fiber during the rising phase of the action potential whereas potassium exit occurs during the falling phase. Restoration of the intracellular potassium, recharging the membrane, then occurs as the increased intracellular sodium ion is reduced to a low level by some as yet unknown mechanism.
Studies of the exchanges of Na\(^{24}\) and K\(^{42}\) between the cell and its environment, Rothenberg (1950), point to a fairly rapid transfer of these ions during activity. Reversal of these rapid ion exchanges is assumed to be effected by the slower metabolic processes which are responsible for transporting sodium out of the cell. This discussion of ion transfer emphasises that complex physico-chemical phenomena are associated with muscle contraction.

There are at present two major theories which attempt to explain the nature of the link between the events of nervous excitation and the muscle contraction.

One, the electrical theory, Lapique, (1936), postulates that the action potential of the nerve impulse upon arrival at the myoneural junction excites the receptor elements on the muscle side of the junction. Through a catelectrotonus effect a potential is produced in this region, called the end-plate-potential. This end-plate-potential need not be completely abolished even though the muscle is paralyzed by d-tubocurarine. When this potential is of sufficient magnitude it fires a chain of events which culminates in a contraction of the muscle. The muscle contraction is always preceded by an electrical activity of the
muscle fiber, distinct from the end-plate potential, which is called the action potential. This sequence of events takes place in a matter of milliseconds.

The chemical theory of the transmission of the nervous impulse across the peripheral or neuromuscular junction Loewi, (1921), gains much support from the observation by Reissem, (1921), that the close intra-arterial injection of acetylcholine evokes a contractile response in striated muscle. Since then numerous experimental findings on the effect of anticholinesterases on the in-situ and isolated nerve-muscle preparations have led some observers to designate acetylcholine as the neurohormone of the neuro-striated-muscular system. This concept of the necessity of acetylcholine in neuromuscular transmission is still in the hypothesis stage.

Recent clinical findings have pointed out that drugs other than known parasympathomimetics or parasympatholytics appear to influence favorably certain disorders of the neuromuscular system. The well known effect of prostigmine in myasthenia gravis has been repeated with other drugs. Partial remissions of symptoms in myasthenia
have been obtained by the use of adrenocorticotropic hormone, (ACTH) McEachern, D (1951). Relief of myotonia by the use of cortisone was reported by the same authors. However, in neither of these diseases have the remissions been permanent, nor as striking as the response of myotonia to quinine. Experienced clinicians have felt that the relief obtained in these lower-motor-neurone disorders were real and due to the drug effects.

In view of these purely clinical observations we feel that a study should be made, comparing the pharmacodynamics of a group of drugs known to have their locus of action on the neuromuscular apparatus. Drugs which appear appropriate for this investigation are prostigmine, d-tubocurarine, quinine, and cortisone.
CHAPTER II

LITERATURE SURVEY OF THE EFFECT OF SELECTED DRUGS UPON THE NEUROMUSCULAR JUNCTION

A. Neuromuscular transmission. The first direct evidence of the cholinergic nature of the motor innervation of striated muscles was obtained by Dale and Feldberg (1934). They demonstrated that electrical stimulation of muscle nerve endings leads to the liberation of acetylcholine in the perfusate of skeletal muscle. Brown, Dale, and Feldberg (1936) later showed that when acetylcholine is quickly injected into the proximate arteries of the mammalian muscle it causes a contraction at almost the same speed and with similar characteristics as a maximal motor nerve stimulus.

In order for acetylcholine to pursue the role of chemical mediator of the nervous impulse several premises must be accepted, Rosenblueth (1950):

(1) That acetylcholine is released at
presynaptic nerve endings and at muscle-nerve endings.

(2) That acetylcholine is released by the nerve impulse in amounts above the threshold of the post-synaptic elements.

(3) That cholinesterase is capable of lowering the acetylcholine value during the refractory period to below the threshold necessary to fire post-synaptic elements.

The chemical theory of synaptic transmission of nervous excitation postulates that a nerve impulse arriving at the terminal arborization of the nerve liberates acetylcholine at the myo-neural junction. This produces an electrical response of the end-plate, called the end-plate potential, which, when of sufficient magnitude, acts to depolarize the adjacent tissue. This series of events leads to a contraction of the muscle fiber. The acetylcholine initially liberated is rapidly destroyed by the cholinesterase present at the
end-plate, Eccles et al (1938). A muscle contraction, in response to a single stimulus, is known as a muscle twitch. The twitch tension will depend upon the number of muscle fibers responding, maximum tension being obtained when all of the muscle fibers respond.

Experimental evidence that the first electrical sign of neuro-muscular transmission is the appearance of the end-plate potential was obtained by Fatt and Katz (1951). When this potential is of sufficient magnitude there may result a wave of depolarization in the muscle fiber. This depolarization wave is known as the action potential of the muscle. The electrical activity travels from the end-plate towards the tendon at approximately two meters/sec. These authors investigated the action of acetylcholine on the myoneural junction and concluded that this chemical produces a large non-selective increase of ion permeability at the end-plate. This "sink" effect allowed not only sodium and potassium ions, but probably all free ions, to cross this membrane, thereby accounting for a potential much larger than acetylcholine alone could produce.
B. Anticholinesterases. The effect of the anti-cholinesterases, especially DFP, and prostigmine, in facilitating neuromuscular transmission, is similar. This was shown by Hunt (1947), and Brown et al (1947). The latter authors used the electromyograph and revealed a prolongation of the electrical activity of the action potential. They classified this as an effect on the action potential of muscle under the influence of these drugs. This effect on muscle of prostigmine is utilized clinically in ameliorating myasthenia gravis. In this disease there is a profound weakness of the skeletal muscles through what appears to be an acetylcholine insensitive state of the myoneural junction. Prostigmine facilitates the nerve impulse transmission and produces a remission. A proper maintenance dosage can ordinarily be found.

When the alkylphosphate DFP was first investigated, it was felt that its more permanent cholinesterase inactivating property would be useful in myasthenia. It was disappointing to find that it was not as effective as prostigmine in this disease, Comroe et al
(1946), and Harvey et al (1947). The explanation advanced to account for this unexpected clinical response was that DFP, contrary to prostigmine does not have, in addition to its anti-cholinesterase property, a sensitizing effect upon the acetylcholine sensitive receptors in striated muscle, Riker et al (1946). This additional action of prostigmine was not shared by DFP nor by eserine.

Brown and von Buler (1938) have shown that in the prostigmine treated animal, the muscle twitch following close intra-arterial injection of acetylcholine, is smaller than the response to the same dose of acetylcholine before prostigmine. We are dealing here with either less sensitivity to acetylcholine in the prostigmine treated animal, or to a blocking effect of acetylcholine at the neuro-muscular junction. In high concentrations acetylcholine has a depressing effect on striated muscle. It is also known that prostigmine decreases significantly the amount of acetylcholine necessary to elicit any of the effects of this mediator on muscle. It appears likely, therefore, that the
action of the injected acetylcholine is potentiated in the presence of an anticholinesterase. Such a block is most probably the result of a persistent depolarization of the end-plate by acetylcholine, in the presence of prostigmine.

C. Curarizing substances. The onset and development of paralysis that follows an intravenous injection of d-tubocurarine is a very interesting pharmacological response, notably because the muscle will respond to direct stimuli but not to an impulse from the nerve. An early study of the electrically stimulated, partially curarized muscle, was performed by Hoffman (1930). With the development of the electromyograph the electrical potential resulting in a muscle following motor nerve stimulation was extensively studied. It was then seen that curarization produced changes in the electrical potentials of the neuro-muscular apparatus. Eccles, et al (1941b) showed that curare diminished the amplitude and duration of the end-plate potential of the muscle fiber without altering its shape or latency. When the electrotonic end-plate potential was reduced to below a certain threshold value no amount of motor
nerve volleys could fire the muscle to contract.

According to Paton et al (1951) muscles which receive their normal stimulus in a tetanic type excitation, like the respiratory muscles, (approximately 20/sec), are especially prone to be affected by a curare-like drug. According to these authors this inhibition is based upon the observation that one of the earliest effects of curare in muscle is an inhibition of the tetanic contraction.

The rapid decay of a tetanus is very pronounced in a curarized muscle. It is more readily obtained in muscles of the "red" type like the soleus. This type of muscle is more susceptible to the action of curare than the "white" type muscle because of the greater ease of fusion of the contractions in the "red" muscle. Some muscles, like the tibialis anticus, are composed of both "red" and "white" fibers, Gordon et al (1940).

The myoneural block by curare in the intact animal is reversible with time. It is also antagonized by such agents as acetylcholine, anti-cholines-
terases, and potassium ions, Wilson et al (1936), and by a new group of substances not possessing anticholinesterase activity, notably the m-hydroxy phenyl alkyl ammonium compounds, Wescoe et al (1951). These last named agents are capable of producing an end-plate depolarization. A tetanizing current is also an effective decurarizing agent, Boyd (1932). The decurarizing action of potassium ions may be due to the release of acetylcholine. The liberation of acetylcholine by intra-arterial injection of potassium chloride has been shown to occur in the sweat glands, tongue, and sub-maxillary glands by Feldberg et al (1935).

The great pharmacological interest and clinical importance of curare-like drugs especially in the field of anesthesiology has led to a careful investigation of the modus-operandi of neuro-muscular block. The antagonism between curare and physostigmine (eserine) was reported by Pal (1900), and Rothberger (1901). The anti-cholinesterase activity of eserine was first suggested by Fühner (1917). This property was later utilized by Loewi et al (1926) to prolong the
action of acetylcholine in their classical experiment 
on the frog heart. Rosenblueth et al (1936) studied 
the effect of neostigmine (prostigmine), a synthetic 
drug, as a decurarizing agent.

Brown, et al (1936) have shown that a dose of 
curarine which produced only a minimal effect on the 
twitch response to nerve stimulation greatly reduced 
the muscle contraction expected from a close intra-
arterial injection of acetylcholine. Here the raised 
threshold to acetylcholine is more apparent in the case 
of exogenous acetylcholine than to the endogenous form.

Grace Briscoe (1938) has stated that in mild 
curarine poisoning, the relation between muscle responses 
to graded stimuli is not altered, while at the same time 
there is a depression of the absolute magnitude of the 
twitch tension. Our results show a somewhat different 
relationship to be the case.

According to Paton and Zaimis (1951) curare 
reduces the end-plate sensitivity to depolarizing drugs 
such as acetylcholine. It is fifty per cent more 
effective in raising the threshold to acetylcholine in
soleus muscle than in tibialis muscle. Brown and Harvey (1938) demonstrated an increase in size of the second of two action potentials from a muscle stimulated by two very close, not more than 150 milliseconds apart, indirect shocks to the gastrocnemius muscle of the fowl. After tetanization, at from 300 to 600 stimulations per second, for ten seconds, the two action potentials from the now potentiated response were exactly equal in size. This indicated to them that, following a tetanus, all the fibers were responding to each of the stimuli. The conclusion that was reached was that in the fowl approximately twenty-five percent of the muscle of the leg fails to respond by contraction to a single motor nerve impulse. The first indirect shock or series of shocks leaves behind a period of raised excitability which lasts about 150 milliseconds. The second impulse then being effective in setting up a contraction of all the muscle fibers.

D. Decurarizing substances. The search for anti-curare and anticholinesterase compounds has produced many congeners of prostigmine. Other compounds,
not related structurally, have been found which possess decurarizing action. Some potent anti-curare drugs are 3-hydroxy phenyl trimethyl ammonium (3-OH TMPA) and decamethonium. Wescoe et al (1951), have shown that 3-OH TMPA and prostigmine produce similar contracture-like responses in directly stimulated denervated muscle.

Decamethonium, a straight chain quaternary nitrogen compound, has interesting pharmacological properties, Paton (1951). The principal effect of this drug appeared at first to be an increase in the sensitivity of the myoneural junction to acetylcholine. This drug is able to decurarize as well as to produce a curare-like block of neuro-muscular transmission. Its action is now thought to be due to its acetylcholine-like action in producing a depolarization of the end-plate. When administered in excess it produces a neuro-muscular block due to a persistent end-plate depolarization. When it follows curare it decurarizes due to this depolarization effect.

E. Post-tetanic effects. Guttman, et al (1937)
investigated the nature of the post-tetanic increase in twitch tension in muscle. Feng et al (1937), (1938), (1939), who did considerable research in this field, also attempted to determine the nature of the post-tetanic potentiation of twitch tension. In a long series of papers they have reported on the effects of tetanus on neuromuscular transmission in the cat. Feng and Li (1941) have reported, using maximal single shocks, that the phenomena of eserine potentiation and post-tetanic facilitation are essentially similar. They interpret both the responses, as being due to a repetitive discharge of the motor nerve endings.

Guttman et al (1937) believed that there was a reserve of energy in the motor unit which was not called forth by a low frequency of stimulation. Furthermore, its availability was limited if called forth early in fatigue. This was shown by a lessening of the post-tetanic facilitation after frequent bursts of tetanus. They also showed that there was an optimum enhancement due to the tetanus provided the tetanus was not of long duration. They localized the phenomenon of post-tetanic
enhancement in the myoneural junction by showing the absence of any post-tetanic effect following direct stimulation of the completely curarized muscle.

In attempting to explain post-tetanic facilitation, Feng et al. (1939) stated that the potentiation which followed tetanus and eserine were alike in every detail. They felt that the conditions brought about at the myoneural junction by a tetanus were steadily maintained by eserine and that whatever mechanism was proposed for one should hold for the other. Dun et al. (1940) showed that the eserine potentiation of contraction in amphibian muscle is different from that in mammalian muscle, the retrograde discharges from mammalian motor nerve endings under eserine being absent in amphibian muscle.

Feng and Li (1941) differentiated the motor nerve endings from the rest of the nerve fibers on the basis of the ability of the motor nerve endings to respond repetitively with eserine. They emphasized the specialized nature of both parts of the myoneural junction, the motor nerve endings, and the end-plate, or
muscle fiber, portion. They pointed out that the action of eserine in myasthenia was probably dual, one on the cholinesterases, and the other directly on the motor nerve endings where it transforms a simple motor nerve impulse into a repetitive one. This was in agreement with the previously reported effect of another anticholinesterase, prostigmine, Riker et al (1946).

Investigations on post-tetanic facilitation by Li and Ting (1941) showed that the response to close intra-arterial injection of acetylcholine in the period of post-tetanic facilitation was irregular. This was sometimes an inhibition and at other times a potentiation. The response obtained most frequently with acetylcholine injection in the post-tetanic period was an abolition of potentiation. An examination of their data, and a fact which they did not stress, reveals that the inhibitory effect of acetylcholine is obtained following high frequency stimulations, 266 per second. A non-inhibitory effect was obtained after a stimulus of forty-nine stimulations per second.

They also reported that following a series of
eserine potentiated twitches the injection of acetylcholine produced a depression of the muscle response. This inhibiting effect of exogenous acetylcholine was also found to occur during quinine potentiation of the muscle response, Oester (1939).

Walker (1951) has shown that post-tetanic potentiation in rat muscle is abolished on cooling to 29° to 20° C. His experiments on the in situ triceps surae of the rat at normal body temperature (37°C) showed that the height of the action potential usually decreases and seldom increases under conditions that bring about potentiation of twitch tension in muscle stimulated through the nerve. Because there is no good evidence that recruitment of additional fibers or repetitive discharge of fibers occurs in the response to single shocks, he has concluded that the potentiation is due in all cases to an increase of contractile strength in the component muscle fibers.

F. Clinical aspects. The ease of muscle fatigue on sustained effort in myasthenia has its counterpart in the myotonic patient. One of the clinical symptoms of myotonia is a persistent contraction of a muscle
after voluntary, mechanical, or electrical stimulation, or even occasionally following a reflex stimulation. There is an apparent rigidity, a repetitive response of the muscle to a single stimulus, an increased sensitivity to acetylcholine, and an exquisite sensitivity to mechanical stimuli. The tonic-like spasm of the contraction is reduced with repeated movements, but returns on resting the muscle. Bremer (1932) records the action of a myotonic muscle (finger flexion) via the ergographic response, wherein the smooth voluntary movement is facilitated following a prolonged stimulation, and the myotonia returns following a period of rest. Full curarization does not reduce the myotonic response of the muscle to mechanical stimulation, Eyzaguirre et al (1948). Microscopically, there is a definite pathology of the muscle fibers, (Case Records, Massachusetts General Hospital, 1937) some of the fibers in the same section showing atrophy and some hypertrophy with fibrosis and fat infiltration between the muscle fibers. Lindsley et al (1936) showed that the after-contraction of myotonic muscle was accompanied by action potentials.
Up to the present time the most useful drug in the amelioration of this condition is quinine. Others, such as ephedrine, potassium salts, guanidine derivatives, etc., have been less successful in relief of this syndrome. This action of quinine has been thought to be due to its ability to suppress repetitive muscle responses of all types, Kolb et al (1938).

The effect of quinine on muscle has been known since Santesson (1892) discussed its action on the contractility and irritability of muscle. It is only in recent years that its beneficial effect in myotonia was discovered, Wolf (1936). Kennedy et al (1937) showed that prostigmine influences unfavorably the course of myotonia. Shy et al (1950), and Mbachern (1951), reported that four to five days of cortisone therapy completely abolished the repetitive volleys of muscle after single stimuli in myotonia patients. At the present time, this effect of cortisone on myotonia, from the experimental standpoint, is almost completely unexplored. Quinine has also been shown to be useful in reducing the myotonic-like response of muscle in rats treated with 2,4-D1 chlorphenoxy acetate (2-4-D), a
weed killing drug, byzaguirre et al (1948).

In summary, it is apparent that prostigmine, curare and quinine have a pronounced effect on the muscle response to indirect stimuli. In the presence of these drugs the muscle twitch may be depressed or potentiated. The action of cortisone has been reported to be partially quinine-like in myotonia, McKachern (1951). It may produce temporary remission of symptoms in this disease. No research on cortisone has been reported which attempts to determine its effect on neuromuscular transmission in the intact animal. Most clinical reports that are available indicate relief of myotonia and aggravation of myasthenia gravis, Schlezingcr (1952). Grob and Harvey (1952) have reported on the effect of cortisone and ACTH in patients suffering with chronic arthritis. They described the development of myasthenia in a 60 year old woman after prolonged use of cortisone. This was most unusual because the incidence of myasthenia in women is much less than in men and in no known cases have symptoms of myasthenia made their first appearance so late in life.
We have selected cortisone and quinine for investigation in order to be able to compare their effects on muscle. We believe that any similarity in these effects should be detectable by the techniques to be described. As a further elaboration of this study, prostigmine and curare were selected for investigation at the same time because of their specific and well defined effects on the neuromuscular apparatus, and possible antagonism to quinine and cortisone.
CHAPTER III

METHODS AND MATERIALS

The test animal employed was the albino rat. Adults, mostly males, of the Sprague-Dawley strain weighing between 190 and 400 grams were used. The animals were anesthetized with intraperitoneal injections of Dial-Urethane (Ciba), 0.1 cubic centimeters per 100 grams body weight. This anesthetic produced sufficient depth and duration of anesthesia for our prolonged experiments. The trachea was cannulated and the internal jugular vein exposed when necessary.

With the animal lying on its side, a lateral incision was made through the ventral aspect of the skin of the thigh, and the femur and sciatic nerve exposed by separating the biceps femoris and the vastus lateralis muscles. The sciatic nerve was freed from adjacent tissue and placed, without cutting, in the groove of a Harvard-type electrode. The electrode was immobilized by clipping the skin flaps together at the edges of the opening and
firmly pressing the electrode against the femur clamp. The femur was fixed by this clamp to a rigid support. The tendon of the gastrocnemius muscle, with a part of the calcaneus, was then cut and attached to an isometric type, flat phosphor bronze spring lever. The thread from the tendon was attached to the lever arm on the writing side of the fulcrum, one-half centimeter from the axis. The writing arm of the lever was fourteen centimeters in length. The pull of the muscle as it contracted was downwards. The rat's foot up to the heel was put in a piece of rubber tubing, and clamped by a burette clamp to a support.

The twitches of the gastrocnemius in response to motor nerve shocks were recorded on a smoked kymograph drum. Single or repetitive-type shocks from an electronic stimulator were applied, at either a constant intensity, or at graduated values from sub-threshold to supramaximal intensity. Tetanic stimulation was usually produced with a current strength, twice that required for maximal response. A frequency of 100 stimulations per second, applied for a period of thirty seconds was used.
The electronic neuro-stimulator (Lab-Tronic) was capable of delivering any voltage from .01 volt to 100 volts in steps of .01 volts. The duration of the electrical pulse could be varied from one-half millisecond (one-half sigma) to ten sigma in steps of one-half, one, two, five, and ten sigma. In all the indirect stimulation experiments a pulse duration of one-half sigma was used. However, in making a response curve from directly stimulated denervated muscle, various durations of stimulus, up to ten sigma, were used. The neuro-stimulator was monitored by a DuMont oscilloscope and found to produce a saw-toothed type pulse.

In the denervation experiments one centimeter of the sciatic nerve was excised, aseptically, under nembutal anesthesia. The animals were allowed two weeks of convalescence. Degeneration of the nervous connection was assured by attempting stimulation of the muscle through its atrophied nerve stump. Since no response to such indirect stimulus could be elicited, the muscle was prepared for the experiment. Stimulation of the denervated muscle was produced by inserting bare number twenty
copper wires into the exposed gastrocnemius muscle. One wire was inserted into the belly and the other wire into the tendon of the denervated muscle. The skin was then replaced about the muscle and clipped in place by skin clips. The current was led through these two wires for direct stimulation of the muscle. The range of current used for direct stimulation was from ten to 100 volts, in steps of ten volts. As a measure of the presence or absence of the drug action, the contra-lateral innervated limb was set up in the same manner and single shocks were applied periodically. Drugs were injected via the intraperitoneal route, as well as intravenously by the jugular vein, depending upon the needs of the experiment.

In order to study any variation in the action potentials of muscle we made use of the experimental procedure described above, to which we added a method for recording the electrical activity of muscle. A Meditron type electromyograph was used for the study of the muscle action potentials.

The rat gastrocnemius muscle prepared for iso-
metric recording was indirectly stimulated through the intact sciatic nerve by a series of graded electrical stimuli which produced twitch tensions from weak sub-maximal through maximal response. Two number four sewing needles insulated except at the extreme tip and separated approximately two millimeters were inserted into the belly of the gastrocnemius muscle. The action potentials of the muscle contractions were led off by means of the needle electrodes. If the action potential obtained was not a smooth diphasic one the electrodes were reinserted or moved until the simplest wave form was obtained. This indicated that there was being recorded the electrical activity of only one or at best a small number of synchronously firing muscle units. The electrical activity was recorded by photographing the visual image produced on the cathode-ray tube of the electromyograph. Sweep times of the cathode-ray tube could be varied from ten to two hundred milliseconds per inch. For the single action potentials the usual sweep time was ten milliseconds per inch. For recording during tetanus the usual sweep time was two hundred milliseconds per inch. In
each experiment the electromyograms were correlated with the corresponding muscle twitch responses.

At the start of a typical experiment we obtained a record of the action potentials and the twitch tensions of the indirectly stimulated rat muscle. The indirect shocks were then adjusted to produce a graded series of muscle responses from weak through maximal. The photographs obtained of the cathode ray images were enlarged and carefully measured on a projection screen. This measurement was facilitated by the use of a calibrated grid drawn upon the screen. Alterations in the spike height and the duration of the action potential during the pre and post-tetanic periods were thus determined. All of these experiments were carried out at room temperature.

The drugs employed were as follows; (1) prostigmine methyl sulfate, 1:2000, (Hoffman-La Roche), (2) d-tubocurarine chloride, 20 units per cubic centimeter, (E.R. Squibb & Co.), (3) quinine hydrochloride, USP (Merck), (4) Cortone acetate, brand of cortisone acetate, (Merck & Co.). The neuro-stimulator was a Neuro-Stimulator #N-104 made by Lab-Tronics, Inc. Chicago, Ill.
Forty-six rats were used in the prostigmine experiments, nineteen rats were used in the curare experiments, twenty-three rats were used in the quinine experiments, and twenty-nine rats were administered cortisone for varying periods of time. Seventy-one were used as normal controls including fourteen with denervated gastrocnemius muscle.
CHAPTER IV

RESULTS

A. Response of normal muscle to indirect stimulation. (S, Figure 1). In measuring the response of a muscle to an indirect stimulus, i.e., by the application of electrical shocks to the motor nerve supplying that muscle, we found a series of stimuli of graduated strength to produce a reproducible series of graded muscle responses.

A characteristic curve, representing the alterations in muscle tension following the application of graded indirect shocks, is obtained by plotting the twitch tension on the ordinate versus the applied voltage on the abscissa. This response is represented in Figure 1 by the curve labelled "S". This curve presents the characteristic increase in the response of the muscle twitch with increasing strength of current. The muscle response reaches a maximum value following a sharp inflection in the curve. The maximal response is then continued in a plateau like region, as the stimulus is further increased.

In the in vivo muscle-nerve preparation, such as
Fig. 1  Response of rat muscle to a series of graded single electrical shocks applied to the sciatic nerve.
S- Muscle responses before tetanus.
†'to 10'- Changes in muscle twitch response at indicated minute intervals after tetanus.
we have described, a series of such tension-voltage curves may be obtained every few minutes without any apparent change in the pattern of the responses. The range of the graduated stimuli should be such as to include maximal and several sub-maximal responses.

**B. Effect of tetanus. (Figure 1).** After obtaining a normal graded response curve, the muscle is stimulated with a standard tetanizing current, which we shall define here as that which is produced by an electrical stimulation of 100 shocks per second, applied for thirty seconds, of a current strength approximately twice the voltage necessary to produce a maximal simple twitch response, and having a duration of one-half sigma. Following the application of such a stimulus to the muscle, resumption of the series of graded single shocks, identical with that of the pre-tetanic series, will produce a group of twitch tension responses which reflect the post-tetanic alterations in muscle.

A typical experiment is illustrated in Figure 1. Here are plotted the pre and post-tetanic responses of the indirectly stimulated gastrocnemius muscle of the rat. It
Fig. 2 Response of denervated rat muscle to direct stimulation. Effect on muscle tension obtained with increased duration of single shocks from 0.5 to 10 milliseconds.
is apparent that immediately after the tetanus and continuing for some time thereafter the tension of the muscle twitch, in response to the original series of graded stimuli, is considerably altered.

Post-tetanically, there is an increased response to an indirect stimulus, reflected by an increased twitch tension. In the first one-fourth minute following tetanus, the maximal response of single twitches was increased by approximately 150 per cent of the pre-tetanic level. This potentiation continues, but in decreasing degree for approximately five minutes. After ten minutes, the response of the muscle has returned to the original level, or is only slightly less than the pre-tetanic control level. Some degree of alteration frequently persists up to ten minutes after tetanus. Such a slight decreased response, persisting late in the recovery period of the muscle, is common, but not invariably present.

C. Response of denervated muscle to direct stimulation. (Figure 2). A series of tension-voltage curves were obtained from rat muscle which had been denervated twelve to fifteen days previously. In these animals,
Electrical stimulation of the degenerated sciatic nerve produced no response of the muscle. Stimulation was therefore of the direct type through two bare copper wires inserted into the belly and tendon of the muscle.

Figure 2 shows the response of the denervated muscle to direct stimulation. Several hundred times as much current was necessary to obtain a response of the denervated muscle, which was comparable to that from a similar, innervated, muscle. The plateau effect, such as was seen in innervated muscle, indirectly stimulated, is absent within the limits of our denervated muscle response curve. Neither post-tetanic potentiation nor any other change, such as sensitivity to prostigmin, was found to be present in the denervated muscle. Therefore, the post-tetanic curves are identical with the curves shown. The family of curves drawn in Figure 2, represent the changes in twitch tension obtained by increasing the duration of stimulus from 0.5 sigma to 10 sigma. Increasing the duration of the stimulus beyond five milliseconds produced no important increase in twitch tension. This response of the denervated muscle has points of similarity
Fig. 3 Effect of prostigmine on the muscle response to a series of graded indirect shocks. Minutes after injection noted on curves. (rat gastr.m.)

S-Curve represents pre-injection response.
with the response of the innervated muscle under partial curarization (see Figure 8).

D. **Effect of prostigmine on muscle twitch tension.** (Figure 3). The effect of intravenously administered prostigmine on the muscle response is so rapid that a method which would result in slower absorption, the intraperitoneal route, was used. This mode of administration enables us to follow in detail the gradually increasing effect of this drug on the indirectly stimulated muscle twitch.

Figure 3 is a series of tension-voltage curves recording the time sequence of the alterations in the muscle twitch tensions following a series of graded indirect shocks in a muscle which is gradually being prostigminized. The potentiating effect of prostigmine appears to be uniform throughout the entire range of stimulation used, from weak sub-maximal, to supra-maximal. However, extrapolation of the curves to their origin on the x-axis (point of zero response), shows that they all originate in a rather narrow range on this axis. Since the magnitude of this range is within the experimental error of
Fig. 4 Effect of tetanus on prostigmine potentiated muscle twitch tension.
A- Prostigmine potentiation of twitch tensions.
B- 30 second tetanus
C- Post-tetanic responses
Voltage levels of twitch responses indicated on curves.
such determinations, we consider that this point of zero response is the same for all and has therefore not been appreciably altered by prostigmine.

E. Effect of tetanus on prostigminized muscle. (Figure 4). During the observations on the development of the prostigmine effect, a standard tetanus was interposed. Following such a tetanus, the previously demonstrated prostigmine potentiation of the single muscle twitches was reduced. After some time the muscle gradually approached the pre-tetanic level of potentiated response previously produced by prostigmine. In cases where the prostigmine effect was waning or disappearing, the return of prostigmine potentiation, as would be expected, never reached 100 per cent.

This post-tetanic depression of the muscle twitch in the prostigmine treated animal was an effect which was just the opposite of that seen in the non-prostigmine treated preparation. This depression of twitch tension following tetanus could be repeated as long as the prostigmine potentiation was present. As the prostigmine effect of twitch potentiation and post-tetanic twitch depression
disappeared, there was a return of the normal post-tetanic potentiation of the muscle response. This phenomenon appeared sufficiently interesting to investigate more thoroughly. Figure 4 is a graph of the results of an experiment in which the effect of prostigmine on the post-tetanic twitches was studied. In this experiment control tension-voltage curves were obtained before prostigmine injection. Prostigmine, 0.2 milligrams per kilogram, was then administered intraperitoneally. A series of graded tension-voltage responses were followed for five minutes during prostigmine potentiation. These were then plotted as percentages of the control tensions, Figure 4 A. The standard tetanus was then applied. For ten minutes following this tetanus, a similar series of graded tension-voltage responses were again periodically obtained. These responses were plotted as percentages of the controls and the results are shown in Figure 4 C.

An examination of the curves in Figure 4 C shows that the first group of post-tetanic responses at the various voltages in this preparation, have all been
Fig. 5. Depressant effect of tetanus on post-tetanic muscle twitches in (A) prostigmine treated rat (0.2mg/kg intraperitoneally), and (B) d-tubocurarine treated rat (0.2U/kg intraperitoneally). (Gastroc. m.)
depressed. The response to subsequent groups of graded stimuli followed for various intervals up to ten minutes, show a progressive recovery in twitch tension up to approximately 90 per cent of the pre-tetanic level. From the results of this experiment, it is clear that in the prostigmine-treated animal there exists a definite depression of the muscle response to an indirect stimulus following a tetanus.

F. Duration of post-tetanic depression following prostigmine. (Figure 5, Figure 6). The effect of this anticholinesterase on the post-tetanic twitch tension was followed for the duration of the drug action in the rat. After establishing the normal tension-voltage curve, prostigmine was injected, 0.2 mg/kg intraperitoneally. At approximately ten minute intervals during the effect of the drug, we obtained a series of control responses from the muscle with a series of graded stimuli. Immediately after each group of control responses was obtained, the muscle was tetanized with the standard tetanizing current. At intervals of one-half, one, two, three, five, and approximately eight minutes following the tetanus, we
Fig 6. Changes in the post-tetanic muscle twitch in rat muscle before and after prostigmine, i.p.

A- Before prostigmine
B- 20 min. after prostigmine, 0.2mg/kg, i.p.
C- 40 min. after prostigmine injection.

Volts used indicated on curves.
recorded the muscle response to a similar series of graded stimuli. The kymograph record from a typical experiment is shown in Figure 5.

The post-tetanic twitch tensions were then calculated as a percent of the control twitch tensions in the pre-tetanic period. Any variation from 100 per cent represents the alteration in the response of the muscle due to the tetanus plus or minus any increment of prostigmine potentiation in the animals. For each of the intervals recorded, one-half, one, two, three, five, and eight minutes, such percentage rating was obtained for each graded stimulus. As a control measure, the contralateral gastrocnemius muscle was attached to another isometric muscle lever. It was stimulated at intervals with single maximal shocks, to determine the presence or absence of the prostigmine potentiation effect, in the absence of any tetanus effect, or any fatigue.

In Figure 6, three curves were constructed to show the duration of the depressant effect of prostigmine on the post-tetanic muscle twitch tension. In Figure 6, A, B, and C, curves 1, 2, and 3, represent the post-
Fig. 7 Alteration in the shape of the tetanus curve following injection of prostigmine in the rat (gastr.m.)

At arrow injection of prostigmine, 0.2 mg/kg i.p.

Each tetanus curve produced by the application of a tetanizing current to the sciatic nerve, (100 sti/sec for 30 sec)
Lettering described in text.
tetanic responses at different voltage levels calculated as a percentage of the pre-tetanic tensions. Graphs B and C were made at different times during the presence of the drug. An inspection of these results shows that in the presence of prostigmine, the post-tetanic muscle response at all levels of stimulation is consistently less than in the normal animal.

G. Changes in the tetanic contraction curve in the presence of prostigmine. (Figure 7). We also recorded the changes in the shape of the tetanus curve during the prostigmine action. Figure 7 illustrates, progressively, the pronounced alteration of this tetanic contraction following the injection of prostigmine. The last curve at the right in this figure was obtained shortly before the prostigmine effect had disappeared. Prominent characteristics of the tetanus curve following prostigmine are as follows:

A - a decrease in the maximum height
B - failure of the curve to return to the original height
C - the early appearance of a rapid relaxation
Fig. 8 Effect of i.p. injection of d-Tubocurarine (0.2u/kg) on the rat muscle twitch. Note gradual straightening of the muscle response curve with increasing curarization. S- Response curve before injection of d-Tc.
phase followed by a secondary rise which is not seen in the pre-prostigmine tetanus

D - the gradual disappearance of this early relaxation phase

E - the appearance of another peak in the tetanus curve toward the end of the response

H. The effect of d-tubocurarine on the muscle response. (Figure 8). In order to carry out a detailed study of the slow onset of curarization the intraperitoneal route of administration was selected. The method of graded stimulation was used to determine the effect of d-tubocurarine on muscle twitch tension.

In a suitably prepared rat, a normal graded response curve was obtained for the gastrocnemius muscle. D-tubocurarine, 0.2 units per kilogram, was then injected intraperitoneally. When the action of the drug produced respiratory difficulty the experiment was terminated. At regular intervals during the absorption of the drug a series of muscle responses to indirect stimuli was obtained. The twitch tensions were then plotted in the usual tension-voltage diagram, (Figure 8). From this
Fig. 9 Changes in the post-tetanic twitch response of muscle after d-Tubocurarine, i.p.

A- Preinjection post-tetanic potentiation.
B- Post-tetanic responses 25 min. after dTc, 0.2 u/kg, i.p.
C- Post-tetanic responses 65 min. after dTc injection.

Volts applied indicated on curves.
figure it may be seen that in progressive curarization the response to graded stimuli is converted from a curvilinear response to a straight line response, at least within the range of voltages used. This linear response is in some respects similar to the response obtained with denervated muscle (Figure 2). Of course with denervated muscle, the expected difference in strength of stimulus is seen.

I. Duration of post-tetanic depression in curarized muscle. (Figure 9, Figure 10). In another experiment, of longer duration, after obtaining the normal pre-tetanic and post-tetanic graded responses of the muscle, we injected d-tubocurarine, 0.2 units per kilogram intraperitoneally. At regular intervals thereafter, we obtained a record of the pre and post-tetanic responses. The post-tetanic changes were followed for five minutes at post-tetanus intervals of one-half, one, two, three, and five minutes. The post-tetanic twitch tensions were then computed as a per cent of the control pre-tetanic twitch tensions for the corresponding graded stimuli. The curves in Figure 9 show how the individual
Fig. 10 Curve A illustrates depression of post-tetanic twitch potentiation in rat muscle in the presence of d-Tubocurarine, 0.2 u/kg, i.p. (at zero time). B- A series of myograms from the same preparation showing early effect of d-Tc on muscle tetanus.
muscle twitch responses, following the tetanic stimulation are altered. It is readily apparent from an inspection of these curves that there exists a post-tetanic depression of the muscle response in the partially curarized muscle. This is in many respects similar to the post-tetanic depression of twitch tension seen in the prostigmine preparation. The kymograph record of Figure 5 graphically illustrates this depression of the post-tetanic muscle twitch in the partially curarized rat. The degree of this depression of post-tetanic twitch tension was followed during the course of the curarization and is illustrated in Figure 10 A. The effect of d-tubocurarine on the tetanic contraction is also shown in Figure 10 B.

J. Effect of quinine on muscle response.
(Figure 11). Quinine hydrochloride (Merok) in dosage from twenty milligrams per kilogram to 200 milligrams per kilogram was injected intraperitoneally, and in some experiments intravenously, into anesthetized rats. After one to two hours, the gastrocnemius muscle was subjected to the usual pre and post-tetanic graded stimuli.
Fig. 11 Effect of quinine on post-tetanic potentiation in rat muscle.

Rat A- Two injections of quinine, 100mg/kg, 16 hours previously, and 50mg/kg, one hour before begining of experiment.
Rat B- Quinine 20mg/kg, i.p.
Rat C- Quinine 200mg/kg, i.p.
Volts used indicated on curves.
The per cent alteration in the muscle twitch to a series of graded stimuli applied to the motor nerve after a standard tetanus, is shown in Figure 11. An examination of these post-tetanic changes shows that in the quinine treated animals, the weak sub-maximal, and the intermediate sub-maximal responses of the muscle, behaved differently from the normal post-tetanic recovery course. In the quinine treated animals, the responses to stimuli producing sub-maximal twitch tensions were depressed following tetanus, and often failed to return to the pre-tetanic levels. Failure to maintain a tetanus was also observed during the period of quinine effect.

K. Effect of cortisone administration on muscle response. (Figure 12). A group of rats was injected daily for various periods of time with cortisone. The dose varied from one milligram per kilogram, per day, per rat, to five milligrams per kilogram, per day, per rat. After courses of treatment from three to eight days duration, they were subjected to the pre and post-tetanic muscle response test. The post-tetanic course of the responses, calculated in the same manner as those repre-
Fig. 12 Effect of cortisone injections on post-tetanic twitch potentiation in rat muscle. (gastr.)
Rat A- 8 daily injections, 3 mg/kg i.m.
Rat B- 3 daily injections, 3 mg/kg i.m.
Rat C- 5 daily injections, 3 mg/kg i.m.
Volts applied indicated on curves.
sented in Figures 6, 9, and 11, is shown in Figure 12. An examination of these graphs shows that the post-tetanic course of the muscle response in these animals was different from the normal. There is a definite delayed return of the submaximal responses to pre-tetanic levels. This depression is not so pronounced in rat A who appeared more refractive to the treatment. The tetanus curve was not significantly altered. There was no pronounced inability to maintain a tetanus. We did observe that under high dosage treatment, five milligrams per kilogram, or more, per day, there was an occasional death. The animals moved about less than the normal rats and appeared partly catatonic.

L. Effect of tetanus on the action potential of normal rat muscle. (Figure 13, Figure 14, Figure 15). After obtaining a record of the action potentials of a series of responses of the muscle to graded stimuli, a tetanizing current of 100 shocks per second, at twice maximal strength, and of thirty seconds duration, was administered. At definite time intervals after cessation of the high frequency tetanizing current, a similar series of
Fig. 13 Changes in the amplitude (A) of the action potential in normal rat muscle (gastr.) following tetanus.
graded single shocks was again introduced.

From photographs of the cathode ray images, two characteristics of the action potential were measured, one, the spike height, and two, the duration of electrical activity. These two variables were each plotted as a function of either the stimulating current, or of the time following tetanus.

Figure 13 is a graph of the pre-tetanic electrical activity of the muscle in response to a series of graded indirect shocks, as well as the electrical activity at one, three, and five minutes following a tetanus. The most striking response for several minutes following the tetanus, is the depression of the spike height throughout the stimulus range. Recovery usually occurred in five minutes.

Further inspection of Figure 13 reveals that in the pre-tetanic period the variation of the spike amplitude with increasing stimulus approximates in form the increase in muscle tension as seen in the curve S of Figure 1. Rosenblueth et al (1940) have shown that this increase in the spike amplitude of the action potential is
Fig. 14 Changes in electrical activity associated with muscle twitches following tetanus in normal rats, in cortisone treated rats, 3mg/kg/day, and in rats injected with quinine, 20mg/kg, i.p. Twice supramaximal stimuli applied to the sciatic nerve.
in linear relationship to the increase in muscle tension.

From Figure 14 it may be seen that in the normal animal the duration of the total electrical activity, RT, undergoes a slight alteration following tetanus. There is a slight decrease in duration of the action potential immediately after tetanus, followed by a rise to slightly above the control level.

From Figure 13, and Figure 14, it is apparent that the effect of tetanus is greater on spike amplitude (A) than on duration of electrical activity (RT). The cathode ray tube trace of the action potentials from normal rat muscle is shown in Figure 15. The muscle was stimulated with a twice maximal response current through its motor nerve at frequencies of twenty-five, fifty, and 100 stimulations per second. Note the sustained amplitude of the action potential.

M. The effect of prostigmine on the action potential of rat muscle. (Figure 16). The effect of prostigmine on the myographic response to single indirect shocks to the gastrocnemius muscle of the rat is to pro-
Fig. 15 Action Potentials from normal rat muscle (gastroc.). Indirect stimulation. (Dial-Urethane anesthesia)

A- 25 stimulations per second.
B- 50 " " "
C- 100 " " "
duce a sharp increase in twitch tension. This increase is soon reduced to a level somewhat lower than the peak response and is represented on the kymograph record as a maximum peak tension within the first few minutes after administration of the drug.

In Figure 16 is shown the electrical activity and the representative mechanical response of the rat gastrocnemius muscle to a series of graded indirect stimuli following treatment with prostigmine. After administering prostigmine, 0.2 mg/kg, intraperitoneally, and continuing the stimulation by the application of maximal indirect shocks to the motor nerve at the rate of one per second, we obtained a photographic record of the accompanying action potentials of the muscle. Variations in the form of these action potentials are graphed in Figure 16. In this figure, curve A represents the spike amplitude, curve RS the duration of the first diphasic action spike, and curve RT the total duration of electrical activity.

From an examination of these curves there appears to exist a correlation between the increase in muscle
Fig. 6 Changes in rat muscle action potentials in response to single supramaximal stimuli after prostigmine, 0.2 mg/kg, i.p., and after 30 second tetanus.

Concomitant diagram of muscle twitch response directly below curves.
tension and the increased duration of electrical activity (RT). This prolongation of electrical activity appears to take place at the expense of the spike amplitude, (A). An interesting aspect of the response is that the duration of the first diphasic spike, (RS), is practically unaltered throughout.

When the prostigmine potentiation reached a maximum and steady state, a tetanus of the type standardized in our experiments was interposed. Single maximal shocks were again applied to the motor nerve and the action potentials of the muscle were recorded. These indirect single shocks were repeated at regular intervals for five minutes.

In Figure 16 the alterations in the action potential following a tetanus are plotted. The total duration of electrical activity, (RT), is decreased, whereas the spike height, (A) is increased in amplitude. In three minutes both increase in magnitude to a maximum and then fall slightly. Curve RS, which represents the duration of the first diphasic action potential, is still of constant magnitude.
Effect of quinine on the muscle action potential in the rat (gastr. m.).

A, B: Electrical responses to indirect stimulation 15 minutes after quinine 50 mg/kg, i.p. (100 sti/sec, at cathode sweep times of 30 msec/inch, A, and 200 msec/inch, B).

A', B': Electrical response one hour after quinine injection. (Stimulation frequency and sweep times same as in A B, calibration as indicated.)
The most significant change in the electrical response following a tetanus, appears to be a depression of the total duration of electrical activity followed by a significant rebound to pre-tetanic levels. This was still undergoing change at the end of the five minute observation period.

N. The effect of quinine on the electrical activity of rat muscle. When quinine is administered to a rat in the dose of twenty mg/kg, intraperitoneally, the effect is to depress the amplitude of the action potential of single muscle twitches. Larger doses depress the action spike still further and when the dose of quinine reaches a toxic level the spike has been depressed a total of about eighty per cent.

The effect of a tetanus on the muscle of the quinine treated rat is a prolonged depression of the action potential of muscle twitches, Figure 14. Post-tetanic recovery of the spike amplitude is very slow in the quinine treated animal.

In Figure 17, A, B, is shown the effect of quinine in altering the muscle action potential during
Fig.18 Effect of chronic cortisone treatment on the muscle action potential in the rat (gastr.m.)

Action potentials during tetanus at 100 sti/sec through the sciatic nerve.

A- Rat 7-19-52, five daily injections
B- Rat 7-17-52, " " "
C- Rat 7-16-52, " " "
All dose levels at 3mg/kg/day.
D- Normal rat
high frequency stimulation. Further absorption of the drug depresses still further the ability of the muscle to maintain its electrical activity during tetanus. (Figure 17 A' B').

0. Effect of daily cortisone injections on the electrical response of rat muscle. (Figure 14 and Figure 18). A series of rats treated with cortisone, three mg/kg intramuscularly, daily, for from five to ten days were subjected to the standardized experimental procedure of pre and post-tetanic indirect stimulation of the muscle. The amplitude (A), and duration (RT), of the action potentials of the muscle were determined for the pre and post-tetanic periods. The averages for the one minute, three minute, and five minute post-tetanic intervals were obtained and the results are shown in Figure 14. This graph shows the delay in recovery of the depressed action potentials after tetanus in the cortisone treated animals as compared to the recovery course of the action potential in the normal animal.

Figure 14 shows the almost complete recovery of
the post-tetanic depression of the action potential in the normal animal to pre-tetanic levels within five minutes.

This comparison demonstrates that a tetanus depresses the spike height and duration of the action potential in the cortisone treated animals to a greater extent and for a longer period than in the normal animal.

In Figure 18 is shown three cathode ray tube traces of the electrical response of rat muscle to a standard tetanus in animals which had undergone cortisone injections, three mg/kg, daily, for five days. The inability of the muscle to maintain the amplitude of the action potential is evident. These responses should be compared with the responses obtained from normal animals, Figure 18 D.

P. Effect of quinine, prostigmine, and d-tubocurarine on the muscle action potential during tetanus. (Figures 17, 19, and 20). In the previous experiments the effect of various agents on the muscle twitch and the electrical activity of muscle were de-
Fig. 19 Muscle action potential responses with increasing frequencies of indirect stimulation in the presence of prostigmine, 0.2 mg/kg, i.p.
From top to bottom, 10, 20, 30, 50, 100, and 200 stimulations per second.
terminated during the pre and post-tetanic periods. An observation which we feel is of great interest is the effect of the above agents on the electrical response of muscle during the early period of rapid repetitive stimulation.

A constant and specific finding during the period of action of these three drugs was the observation that during stimulation with a frequency of 100 stimulations per second the action potential of the muscle was reduced to almost zero level after the first few shocks. This rapid depression of the electrical activity during tetanic stimulation was correlated with the myogram responses which showed a marked inability of the muscle to maintain a contraction.

If one examines these muscle action potentials of the first few milliseconds during the application of a tetanizing current to the motor nerve an interesting phenomenon is seen. The diphasic spike response to the first stimulus may have a normal amplitude which is, however, progressively reduced in the succeeding shocks until, by the fourth or fifth shock, its amplitude may be reduced to almost zero levels.
Fig. 20 Muscle action potential responses with increasing frequencies of indirect stimulation during partial curarization.

From top to bottom: 20, 50, 100, and 200 stimulations per second.
CHAPTER V

DISCUSSION

In determining the effect of pharmacological agents which have a locus of action on muscle it is generally considered that supra-maximal stimuli produce the most useful form of muscle response. We have shown by the reproducibility and the regular character of the tension-voltage response curves that a graded series of voltages is suitable for following alterations in muscle response and will provide considerably more information than supra-maximal stimuli alone.

We have demonstrated, with the method of graded stimuli, that it is possible to follow, in a roughly quantitative manner at least, alterations in the response of indirectly stimulated muscle.

For purposes of discussion and ease in handling the data we have divided the response of muscle into three areas, the weak sub-maximal, the moderate sub-maximal, (roughly 75 per cent of maximal) and the maximal response areas. These regions, we believe, cover the important response patterns of muscle. We have implemented
the study of these patterns by setting up families of
tension-voltage curves. These curves represent the re-
sponses of muscle from the weak sub-maximal region, through
the area of maximal response.

An illustration of the sensitivity of this tech-
nique is shown by our experience with the effect of sever-
ing the central connection of the sciatic nerve. If the
rat gastrocnemius muscle is stimulated at regular intervals
with a series of single graded electrical shocks through
its intact motor nerve, the sciatic, the responses of the
muscle quickly reach maximum values. The tension produced
by the muscle at the various levels of stimulation may then
be plotted as points on a sharply rising curve. This series
of single graded shocks to the motor nerve may be repeated
every few minutes during the course of several hours without
significant alteration of the response pattern. If now
the sciatic nerve is cut between the point of contact of
the stimulating electrode and the spinal cord, the subse-
quent muscle twitches will show an immediate alteration in
response. These changes, for the most part are an increased
sensitivity to low threshold stimuli and are therefore most
noticeable in the pattern of the responses to sub-maximal
stimuli. This change in the tension-voltage response curve of the muscle indicates an altered threshold of response to an indirect stimulus. If we follow the tension-voltage changes during several hours following nerve section we will note that these curves, especially the responses to sub-maximal stimuli, take on a new configuration which reflects this altered sensitivity of the muscle. The change in response is usually one of decreased threshold, tending to shift the muscle response curve to the left in Figure 1, i.e., toward the region of decreased stimulus current. With supra-maximal shocks this shift of the sub-maximal response is not ordinarily apparent. These important alterations in the overall response of the muscle following severance of the sciatic nerve led us, in order to simulate as closely as possible normal conditions, to make use of the preparation with the nerve in continuity with the spinal cord rather than the sectioned nerve preparation.

In the main our experiments, where they are similar to the previously reported work, have reaffirmed the previous findings on the course of the post-tetanic facilitation in the nerve muscle preparation.
A. Effect of tetanus in the normal animal. Our experimental results with the phenomenon of post-tetanic facilitation are charted in Figure 1. This figure is a series of curves representing the course of the normal post-tetanic response in the gastrocnemius muscle of the rat by means of a series of graded stimuli. From an examination of this particular data the following observations may be made: (1) twitch potentiation after tetanus is greatest with a supra-maximal stimulus, (2) responses to sub-maximal stimuli are first depressed and then slowly potentiated, (3) when the potentiated responses to supra-maximal stimuli have begun to subside, the responses to sub-maximal stimuli are still being potentiated.

When considering the onset of effect, rate of recovery, and the course of the facilitation phenomenon, in normal muscle, the above observations point to a dual nature of post-tetanic facilitation. In the normal animal, post-tetanic potentiation does not uniformly facilitate the response to all strengths of stimuli but more slowly potentiates responses to sub-maximal stimuli. The muscle twitch tension is uniformly elevated, especially to stimuli at the maximal level. The duration of these changes following
a tetanus, as studied in the response of a muscle to single stimuli applied to the motor nerve, may persist up to ten minutes. Such a long lasting effect indicates that more than a simple increase in acetylcholine has occurred, since any excess acetylcholine would be expected to be readily hydrolyzed by the esterase present in the tissue. It is most likely that other functional changes, in either receptors, or motor nerve endings, or both, may take place following a tetanus.

Speculation on the origin of the alterations in muscle twitch tension following a tetanus leads us to enumerate a few of the possible mechanisms by which these changes may be mediated:

1. Increased production of acetylcholine at motor nerve endings.
2. Inactivation or inhibition of cholinesterase.
3. Facilitation of neuro-muscular transmission through altered thresholds.
4. Alteration of muscle sensitivity.
5. Increased contractility of muscle substance.
6. Recruitment of muscle fibers.
7. Asynchronous firing of muscle fibers.
(8) The ionic effect of potassium-sodium transfer.

The role of mechanisms numbers four and five, which are seen to be peripheral effector cell changes, should be presumed minimal because of our observation that post-tetanic facilitation is absent in denervated muscle.

Walker (1951) believes that muscle contractility may be increased following a tetanus. If this be so it must be mediated through some neural mechanism.

Potentiation of the mechanical response of a muscle following a tetanus is apparently produced only in the presence of a non-degenerated neuro-muscular system. Indirect evidence in support of this theory is the failure of denervated muscle in our experiments to produce a post-tetanic increase in muscle response to direct stimulation, as well as the inability of an anticholinesterase, prostigmine, to potentiate the response of denervated muscle.

Direct tetanic stimulation of muscle which retains its normal innervation, results in the typical post-tetanic potentiation of the muscle twitch.

At present the mechanism of these post-tetanic alterations is unknown. Within limits, however, we can state that the electrical changes in our preparations have
persisted for periods about equal to the duration of the myogram changes.

The maximal post-tetanic potentiation of rat muscle is paradoxically accompanied by a depression of the amplitude of the action potential. These anomalous responses can be brought into conformity only when we realize that the process of depolarization of a muscle fiber implies a breakdown of the semi-permeability of the membrane. The movement of potassium ions from their intracellular site to the outside of the muscle fiber is effected at almost the same time that sodium ions travel inwards. We have observed during a thirty second tetanus that the bio-electric phenomenon, the muscle action potential, is considerably reduced in amplitude at the end of the stimulation. Might not this transfer of ions in both directions produce sufficient concentration changes to account for the observed electrical and mechanical alterations of the muscle response on an inotropic basis?

A positive answer to this question would have to take into account the observation that denervated muscle, at least 10 days after section of its motor nerve, is no longer susceptible to post-tetanic potentiation.
We have observed that section of the motor nerve during stimulation produces at once wide alterations in the sub-maximal response region of the muscle twitch. Again we ask, may not the nervous continuity be important for maintaining the bioelectric membrane potential at predetermined high levels?

We have also observed that anoxia produced by closing the trachea of the rat may yield an increase in the muscle twitch tension in the sub-maximal response region. This response is interesting, since it has been reported that depolarization of nerve by potassium liberation may be produced during anoxia, Shanes and Brown (1942), and Gerard (1930).

B. Electrical activity in normal rat muscle. In the response of normal rat muscle to increasing strength of indirect stimulation, it was observed that the electrical spike height varied in a parallel manner with muscle tension. The total duration of electrical activity, however, was only slightly altered during the pre-tetanic series of graded shocks. After tetanus the following changes in the action potential were noted: (Figures 13 and 14,), (1) spike height was depressed throughout the range of stimulation,
(2) recovery of the spike amplitude was practically complete in five minutes, (3) the total duration of electrical activity during maximal stimulation, Figure 14, was slowly increased to approximately 15 per cent more than the pre-tetanic level. The period of time involved in the recovery from the tetanus, indicated by the slow return to normal of the electrical phenomena, leads us to believe with others that changes in the concentration of ions at the surface of muscle fibers may be responsible for the potential changes observed. The most likely ions involved being potassium and sodium ions.

Brown and Nuhler (1938) have shown that this diminution in amplitude of the action potential in mammalian muscle following tetanus is probably due to a depolarization of the mammalian muscle fibers and not to a decrease in number of muscle fibers responding.

It appears from the above findings, and from the findings of others, that following a tetanus some alteration of chemical mediators, either through an increase or decrease in their quantity, at the surface of membranes or in cells may occur. These changes may lead to an alteration in the response threshold of myoneural or muscle receptor elements thereby producing an apparent alteration in muscle
sensitivity.

C. **Effect of prostigmine.** Some of the prominent well known effects of prostigmine on the muscle response in the intact animal are, a potentiation of the twitch tension, an inability to maintain a tetanus, and a repetitive firing of the muscle action potential in response to a single stimulus. 

An examination of the family of curves in Figure 4 demonstrates the depressant effect of a tetanus on the subsequent muscle twitches in the prostigminized animal. In addition, Figure 4 A, these curves appear to indicate that prostigmine potentiates the responses to certain strengths of stimuli to a greater extent than others. However, this may be an artifact due to the geometry of the tension voltage curves.

In another type of experiment the degree of depression of the post-tetanic response following prostigmine was correlated with the amount of drug effect present. In Figure 5 the course of the post-tetanic depression of the muscle twitch during various levels of prostigmine effect was followed and a graph of the results is given.

Both prostigmine and tetanic stimulation result
in a potentiation of twitch tension. The family of curves in Figure 3 which depict the alteration of the muscle twitches under the potentiating action of prostigmine should be compared with the responses obtained during the potentiation period following a tetanus in normal muscle, (Figure 1). A tetanus has a variable effect on the muscle response depending upon the strength of the individual twitch stimuli whereas prostigmine potentiates all muscle responses, whatever the strength of stimulus.

We have shown, as well as others, that when a tetanus is introduced during the period of prostigmine potentiation of the muscle twitch, that the succeeding twitches in response to single shocks, instead of being further potentiated, as in the normal animal, will be depressed. Return of the potentiated response usually occurs within five minutes. Thus there are at least two dissimilarities between the potentiation of twitch tension which follows a tetanus and the potentiation of the muscle twitch which occurs following prostigmine injection.

D. Effect of prostigmine on electrical activity of muscle. Prostigmine produced an immediate decrease in the amplitude of the muscle action potential spike while
at the same time the duration of electrical activity was considerably increased, (Figure 16). After the prostigminized muscle had been subjected to a tetanus, resumption of maximal single shocks was accompanied by an increase in spike height and a marked reduction of duration of the electrical activity. The duration curve (RT) rebounded in three minutes to a higher value and then followed closely the slow fall of the spike amplitude.

An important observation in the prostigmine treated rat is that there appears to be a relationship between the muscle twitch tension and the total duration of the action potential of the muscle.

It is evident that in the prostigmine treated animal the increase in twitch tension is well correlated with total electrical activity and not correlated with spike amplitude.

Another important finding during the course of the prostigmine effect is that the duration of the first diphasic action spike is unchanged throughout the wide alterations undergone by other parts of the action potential. This leads us to believe that propagation of the bioelectric potential of the muscle fiber is not altered in the presence
of prostigmine.

E. Effect of d-tubocurarine. Following the injection of d-tubocurarine, the tension-voltage curves of the muscle, in response to graded stimuli, show the onset of a characteristic depression of the muscle response, Figure 3.

An examination of this family of curves indicates that d-tubocurarine may exert a selective effect on factors affecting muscle response. The rapid recruitment of muscle fibers, as seen in Figure 1, necessary to produce a steep gradient of rise in muscle tension with increasing strength of stimuli is the first response inhibited. The responses to weak sub-maximal stimuli are also the earliest to disappear. The final set of readings taken in the almost completely curarized animal shows that the residuum of the contraction resulting from the graded stimuli, as we approach complete curarization, may be plotted along a straight line. This response is seen to be quite similar to that obtained from denervated muscle, (Figure 2), in which the rapid recruitment of muscle fibers is inhibited and the muscle tension is a direct function of the stimulating current. A low gradient of rise in muscle tension with increasing strength of stimulus is therefore a characteristic feature
of denervated and partially curarized muscle.

An interesting observation in the partially curarized animal is a post-tetanic depression of the muscle twitch similar to that seen in the prostigmine treated animal, Figure 5. This is an unusual finding in view of the fact that these drugs have diametrically opposite effects on muscle tension in the non-tetanized muscle. This post-tetanic depression of the muscle response in the partially curarized animal may be the result of one or more of the following mechanisms: (1), a depressed sensitivity or alteration in the activity of muscle receptors to acetylcholine, (2), decreased liberation of acetylcholine by the motor nerve endings, (3), exposure of new receptor sites thereby increasing the net curarization effect.

The effect of d-tubocurarine on the muscle action potential may be seen during indirect, rapid repetitive stimulation of the partially curarized muscle, Figure 20. Within the first three to four shocks during tetanus, the action potential is reduced to almost zero value.

F. Effect of Quinine. The muscle of the quinine treated animal is unable to maintain a tetanus. There is
also a concomitant inability of rat muscle to maintain the amplitude of the muscle twitch action potential after quinine treatment; also during and following indirect high frequency stimulation, the action potential is depressed in the quinine treated animal, Figure 14 and 17. This effect of quinine in depressing the mechanical response of muscle, as well as the action potentials, is in accord with its effect in myotonia. In this lower-motor-neurone dys-function it exerts a definite anti-myotonic effect.

In the rat under quinine the effect of interposing a tetanus during a series of single sub-maximal shocks is to produce a long lasting depression of the muscle twitch, Figure 11. The responses to weak sub-maximal, and to moderate sub-maximal stimuli are markedly depressed in the early post-tetanic period, Figure 110. In contrast, the maximal responses may be potentiated, but not as much as in the absence of the drug.

G. Electrical response following high frequency stimulation. The rapid suppression of the action spike within the first few stimuli during high frequency stimulation following treatment with prostigmine, d-tubocurarine, and quinine is apparently a previously unreported phenomenon.
The mechanical response to tetanus in each instance is shown to be an inability to maintain a tetanic tension. The myograph picture is a single twitch-like response. This single twitch is understandable when the accompanying action potentials are seen to be reduced to almost zero levels in thirty to forty milliseconds. The striking similarity of the muscle response to a tetanus under the influence of these dissimilar pharmacological agents is unexpected and not easily explainable.

H. Effect of cortisone. No experimental reports of the effect of cortisone on the myoneural junction are available.

Ingle et al (1949) showed by muscle work performance tests that the removal or inactivation of one adrenal gland limits the ability of the rat to work. However, continuous intravenous injections of beef adrenal cortical extract enabled adrenalectomized, and adrenal enucleated rats to work as well as rats having intact adrenal glands.

Torda et al (1950) reported on the partial reversibility by cortisone of muscle failure during work performance tests in adrenalectomized animals. They also stated that optimum neuromuscular function is obtained when there is an
equilibrium in the hormones secreted by the adrenals. They did not indicate which of the adrenal hormones were most important.

A recent paper by Torda and Wolff (1952) has demonstrated a depression of the acetylcholine synthesizing ability of nervous tissue following both ACTH and cortisone treatment in experimental animals.

Our experiments with cortisone treated animals using the graded stimuli technique indicates that in animals undergoing long term administration of cortisone there exists a prolonged post-tetanic depression of the muscle twitch in response to an indirect stimulus, Figure 12. This is in contrast to the relatively short post-tetanic potentiation seen in normal muscle, Figure 6A. We have, in addition, by means of the electromyograph, demonstrated that chronic cortisone therapy in rats produces a long continued post-tetanic depression of the muscle action potential, Figure 14. Both the spike and the duration of electrical activity are affected. This is additional evidence in support of our findings by means of the mechanical myogram that there exists a depression of the muscle response in the cortisone treated animals.
Lindsley (1935) has shown a rapid decrease in the action potential of indirectly stimulated, fatigued muscle in man during rapid repetitive stimulation. We have shown, Figure 18, that in the cortisone treated rat, there is a rapid decline in the amplitude of the action potential during application of the standard tetanus. This depression of electrical activity, in response to a high frequency of stimulation is similar to, and may be compared with, the effect of quinine on rat muscle, Figure 17.

I. Molecular basis of post-tetanic effects. The ability of muscle to modify its twitch tension in response to a direct stimulus may be affected by many variables, some of which are, rest, exercise, drug action, etc.; other more fundamental factors are (1) number of muscle fibers responding, (2) rate of stimulation (summation effects), and (3) initial tension. With motor nerve stimulation further variables are introduced some of which are, (1) stimulation of nerve, and (2) transmission of the impulse through the myoneural region.

Ramsey and Street (1941) have shown that in the isolated single muscle fiber the application of a current of rheobasic strength will produce a single muscle twitch.
Increasing the current strength leads to a repetitive response of the muscle fiber. The repetitive response is small or absent at low temperature.

They also observed that a tetanic stimulation produced a preliminary short normal tetanus which was followed by a series of twitch-like responses as the muscle fiber responded to only a few of the repetitive shocks. The enhancement of single twitches after a tetanus seen in their isolated fibers was explained as being due to some property of the muscle fiber and not of the end-plates since they were working with fibers which had been isolated for several days and it was therefore assumed that end-plate degeneration had occurred.

This post-tetanic potentiation may be found in muscle denervated up to ten days according to Brown and Euhler (1938), but was not seen consistently after longer periods of denervation. In our preparations which were denervated for longer periods, post-tetanic potentiation was not seen.

That activity of muscle leads to the loss of intracellular potassium was observed by Reginster (1938). He concluded from work on frog muscle that direct and indirect
stimulation of normal and curarized muscle as well as direct stimulation of denervated muscle leads to the release of potassium from muscle.

Other observations on the effect of potassium on muscle made by Brown and Euhler (1938) showed that close intra-arterial injection of KCl may be followed by potentiation or depression of the motor nerve twitch, depending upon the concentration of the salt, and the frequency of injection.

They also showed that if a tetanus was interposed during the increase in twitch tension induced by small doses of KCl the post-tetanic twitch was potentiated. If a tetanus was administered during the depressant effect of large doses of KCl on single twitches the subsequent post-tetanic twitches were either unchanged or sometimes slightly depressed.

Walker (1947) has suggested that when the twitch to tetanus ratio is low, such as is found during potentiation of the muscle twitch by KCl, post-tetanic potentiation is not seen because the muscle response is already potentiated by the presence of potassium ions in the extracellular fluid. Ramsey et al (1941) also discussed the relation of different
twitch to tetanus ratios of single muscle fibers to post-tetanic changes in twitch tension.

It has been suggested by Hermann et al (1938) that the presence of extracellular potassium may facilitate the liberation of acetylcholine. The release of this substance, acetylcholine, then indirectly potentiating the muscle twitch.

It was shown by Torda and Wolff (1946) that eserine potentiates the muscle response to potassium ions. This observation may aid in explaining some of our results with prostigmine and curare.

Prostigmine, and also small doses of curare, produce a post-tetanic depression of single twitches and an inhibition of a tetanus. This observation suggests that during a tetanus the first effect of curare and prostigmine may be to facilitate the release of potassium ions from the muscle fiber. The excess of potassium in the extracellular fluid or its presence on the outer muscle membrane in the presence of acetylcholine released by the tetanus may exert a depressant effect on twitch tension.

An examination of the effect of prostigmine on the action potential of single twitches, Figure 16, shows a
progressive depression of the amplitude of the electrical response with increasing prostigmine absorption. This response is similar to the known effect of potassium ions in depressing the action potential of muscle contraction. Our data also shows that following a tetanus the amplitude of the action spike in response to single stimuli rebounds to almost normal values. This effect is different from the depression of the action potential seen in normal muscle after tetanus. This return to normal of the action potential following a tetanus in the prostigmine treated animal may indicate that an excess of acetylcholine acts to block the depressant effect of extracellular potassium ions on the resting membrane potential during excitation.

The characteristic response of the normal muscle action potential following a tetanus is illustrated in the graph of Figure 13. There is seen a pronounced depression of the amplitude of the action potential throughout the range of graded muscle response. That this is not due to fewer muscle fibers responding after the tetanus is evidenced by the large concomitant increase in twitch tension.

The most probable source for the diminution of the size of the action potential may be in some alteration
of the resting membrane potential. Experimental evidence that the resting membrane potential is reduced when KCl is injected into a muscle has been reported by Brown (1937). He has also observed that the demarcation potential of muscle fibers was reduced following tetanus, and also following the injection of KCl. This salt was shown to be more potent than a tetanus in reducing the resting muscle membrane potential. KCl also increased the depressant effect on the resting potential with increase in the dose. It was also reported that treatment with large doses of KCl resulted in a depression of twitch tension. Small doses were more effective in producing an increase in muscle twitch tension. A tetanus usually yielded a decrease in demarcation potential. These phenomena are relatively long lasting and most likely due to chemical changes which out-last the stimulation.

It may be pointed out that in our experiments the response of the muscle to small doses of curare appears to be different from the response to large doses, i.e., doses which depress the twitch response almost to zero value.

This dual response of the muscle is apparent when we compare the effect of a tetanus and also the effect of KCl injections in the animal under a complete or almost

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complete curare produced neuromuscular block with the effect of a tetanus on the slightly curarized animal. It is well known that tetanus and KCl will produce a decurarization with an increase in twitch tension. This indicates that the responses of the almost completely curarized muscle are true sub-maximal ones due to the failure of some of the muscle fibers to respond. This also strongly suggests that the effect of these decurarizing agents is on the end-plate region at this stage of curarization.

With small doses of curare some other mechanism must be affected since we have shown that a muscle tetanus during slight curarization yields a post-tetanic twitch depression. We have not found any reports in the literature on the effect of KCl during slight curarization.

Walker (1947) studying the effect of KCl injection, adrenalectomy, and tetanus on the action potentials accompanying single twitches of muscle reported a marked potentiation of muscle twitch tension by KCl without repetitive discharge of the muscle. It was suggested that the action of extracellular potassium is on the muscle fiber membrane, thereby accounting for the increased duration and decreased height of the action potential noted in their work. These
effects on the electrical response of the muscle were believed to produce an increase in contraction time of the muscle fibers and thereby increase twitch tension. An increase in the latent period was also seen which suggested to them that KCl had an effect at the neuromuscular junction. Walker also pointed out that the only known manner in which potassium might potentiate the muscle response was by prolongation of contraction time with a concomitant dispersion of the response of the fibers thus allowing an effective summation of the early responding muscle fibers with those responding later.

Walker (1948) found that a tetanus of 200 stimulations per second induced a slight decrease of action potential height and no change of action potential duration in post-tetanic responses of muscle stimulated for one to two seconds. Tetanic stimulation for two to three seconds at the same frequency induced a greater decrease of action potential height and some increase of action potential duration. Tetanic stimulation for ten seconds brought about a marked decrease of action potential height and a marked increase of action potential duration. He postulated that at the increased frequency of stimulation potassium
accumulates in appreciable quantities in the extracellular fluid immediately surrounding the muscle fibers and thus affects the action potential by its well known effect in depressing the resting membrane potential.

We have shown that this depressed amplitude of the action potential following a tetanus exists at all levels of graded response. It thus appears independent of the stimulus strength and may be an effect of the peripheral fiber.

J. Action of anticholinesterases. In the mammalian nerve-muscle preparation the anticholinesterase, prostigmine, gives rise to several characteristic effects. The muscle twitch of a preparation under the influence of prostigmine in response to a single indirect stimulus is potentiated up to 100 per cent or more. During this twitch potentiation the amplitude of the action potential is reduced, as we have shown, Figure 16. The total duration of electrical activity however, is considerably increased. The diphasic muscle action potential appears to be converted into an asynchronous salvo. This is indicative of a repetitive response of the muscle units to a single nerve stimulus.

Furthermore, excitation of the prostigmine treated
muscle by a train of supramaximal shocks applied to the motor nerve at the rate of 100 per second causes, instead of a sustained tetanus, a twitch like contraction of the muscle followed by relaxation.

In the prostigmine treated animal an excess of acetylcholine produces a paralyzing effect on neuromuscular conduction. This is suggested by the observation that the potentiated responses of skeletal muscle during prostigminization are abruptly depressed by the intra-arterial injection of small amounts of acetylcholine, Brown, et al (1936).

The depression of twitch tension produced by exogenous acetylcholine and the post-tetanic depression of single twitches noted in our prostigmine treated animals may be related if we consider that in the presence of an anti-cholinesterase an indirect tetanic stimulation of the muscle may lead to the accumulation of an excess of endogenous acetylcholine at the end-plate.

The tension-voltage curves of rat muscle following the administration of prostigmine show that this drug greatly enhances the sensitive component of the muscle response. (Figure 3)
There appears to be no change in the threshold to the electrical stimulus while at the same time the twitch tension is potentiated. This increase in twitch tension is very probably, in part at least, the result of an accumulation of acetylcholine at the end-plate.

The efficacy of prostigmine in the myasthenic may be due to its effect in increasing the sensitive phase or neurogenic component of transmission. Buchtal and Engbaek (1948) have shown that the threshold to intra-arterially injected acetylcholine is considerably increased in the myasthenic, approximately five fold. The administration of prostigmine reduces the quantity of acetylcholine necessary to obtain a minimal muscle response. An examination of myasthenic muscle in situ by the method of graded response may provide interesting information on the nature of the muscle depression present in this disease.

Recently Zaimis et al. (1952) have shown that decamethonium may not be acting at all times as a substance which produces neuromuscular block through a persistent depolarization of the motor end-plate. In some species of animals, monkeys, dogs and hares, it is shown that this drug produces a block by competitive inhibition.
In spite of the similarity of the pharmacological response to acetylcholine in these species it is suggested that there must be distinct physical differences in the muscle membrane. Furthermore, these changes may be evident in other species, e.g. man, as pathological changes in the muscles. In this study by Zaimis the action of decamethonium in the myasthenic is shown to produce a neuromuscular block which is antagonized by anticholinesterases. It is suggested that the sensitivity of the motor end-plates in the affected muscles of the myasthenic is altered.

This report supports our view that two types of muscle responses may be elicited by drug action depending upon the ability of a drug in blocking what we have called the sensitive or insensitive components of muscle contraction.

K. Action of d-Tubocurarine. Little is known of the biological action of curare on the molecular level. It is generally believed that its action on the tissues can be represented by the following reversible reaction.

\[
\text{Curare} + \text{Receptors} \rightleftharpoons \text{Curare-Receptor complex.}
\]

No implication as to the nature of the receptors is made in this reaction. The degree of block being related solely to the number of receptors occupied. The evidence for the
above reversible combination is based on the following observations:

(1) The action of curare is reversible with time.
(2) The stability of onium ions as opposed to the easily broken ester linkage of acetylcholine contributes to the reversibility of this complex.
(3) In the isolated nerve-muscle preparation the degree of neuromuscular block is dependent solely upon the rate of diffusion of curare into and out of the preparation.

Ing and Wright (1933) have postulated that the onium ions of curare by virtue of their properties as stable monovalent cations, similar to the alkali metals, may replace sodium or more likely potassium ions, at the motor end-plate. The onium ions however, being unable to fulfill the function of the displaced ions.

In our experiments we have shown that the earliest effects of curare in rat muscle are, (1) an inhibition of the tetanic contraction in response to an indirect rapid repetitive stimulus, (2) a reversal of post-tetanic potentiation of twitch tension, and (3) rapid suppression of the
muscle action potential during an indirect tetanus.

If we examine the family of tension-voltage response curves of muscle obtained during increasing curarization in the rat, Figure 8, it appears that curare at first blocks what we have called the "sensitive" component of muscular contraction. This component of the muscle response is represented in our graded response curve by the quick contractile element of the innervated muscle. Increasing the degree of curarization converts this quick contractile response of muscle into what we will call the "insensitive" fraction of muscle contraction.

The muscle twitch-tension during this insensitive stage is a linear function of the applied stimulus. The tension voltage response curve of the muscle now resembles that obtained from denervated muscle, Figure 2.

The progressive blocking by curare of the quick component of the muscle twitch and the conversion of the response into a second or insensitive type of contraction is an important finding made with the method of graded response.

Buchtal, et al (1942) and Engbaek (1948) have postulated that depending upon the concentration of curare,
the first boundary of the motor end-plate, which they call
the neurogenic component, may be blocked, while the excita-
bility of the second boundary, the so-called myogenic
component remains unaltered. Further curarization blocks
both components of the myoneural junction.

The conversion of a tetanus into a twitch-like
response early in progressive curarization may be due to the
selective blocking of the sensitive components of neuro-
muscular transmission.

This component being represented in the tension-
voltage curve as the quick contractile region. Conversely,
the tendency of certain muscles to respond to a single
stimulus with a brief tetanus instead of a single twitch,
such as occurs in myotonia, may be the result of an excess
activity of the sensitive component in myoneural transmission.

In the myasthenic the effect of small doses of
curare is to produce a pronounced muscular weakness. The
potentiated action of this drug in this disease may be
accounted for if this sensitive component of neuromuscular
transmission could be shown to be subnormal.

In the partially curarized animal at the level
of curarization which is just sufficient to convert a tetanus
into a twitch-like response, the effect of a thirty-second tetanus was to change the post-tetanic potentiation of single twitches into a post-tetanic depression.

This blocking action on post-tetanic potentiation by a slight degree of curarization may be explained on the basis that since only a certain proportion of receptors are blocked by curare, there is an amount of acetylcholine in excess of the normal amount available for the unaffected receptors. This explanation is not opposed to the well-known effect of a tetanus in decurarizing more deeply curarized preparations.

It has been found in the present study that the ability of the partially curarized muscle to maintain the action potential during rapid indirect stimulation is markedly reduced. When a train of impulses reach the myoneural junction, a certain number of these, two or three, will pass the junction before block occurs, and fire the muscle to contraction, Figure 20. The first of the train of impulses into the myoneural region is transmitted beyond this point. The muscle responds to this first stimulus with a normal action potential. The second and third electrical responses of the muscle reveal a progressive and marked
reduction in spike amplitude indicating that transmission of the nerve volley is reduced. Further impulses produce a negligible electrical response of the muscle, thus indicating that for all practical purposes an almost complete block in neuromuscular transmission has occurred.

Apparently when stimuli pass the myoneural junction in normal muscle the receptor mechanism in the interval between the stimulations is rapidly restored to pre-stimulation levels. Curare appears to depress the speed of this restoration.

The effect of even small doses of curare on the tetanic contraction of muscle to an indirect iterative stimulation is thus somewhat clarified. There occurs a rapid suppression of the action potential of muscle with a complete block of myoneural transmission taking place in a few milliseconds. The tetanic contraction is aborted and appears as a twitch-like response followed by complete relaxation of the muscle. With complete curarization the action potential is completely suppressed, only the end-plate potential is apparent.

L. Action of quinine on muscle. Dawes (1946) has pointed out that substances which prolong the refractory
period in the heart, such as procaine, quinidine, and quinine, are substances which depress the action of acetylcholine in many different tissues. The effect of quinine in lengthening the least effective interval for the summation of two successive stimuli in skeletal muscle has been demonstrated by Oester (1939).

Our experimental observation on the effect of quinine in the response of rat muscle has shown that a rapid repetitive indirect stimulus produces within a few milliseconds a pronounced inhibition in the amplitude of the muscle action potential. Rapid suppression of the electrical response to a repetitive stimulus in the presence of quinine is also reflected in the inability of the muscle to maintain a tetanic tension. There is present a depression of the electrical responses of single twitches and a further depression of this electrical activity follows stimulation of the quinine treated muscle by a tetanus, Figure 14.

Other studies indicate that quinine may have an effect on the muscle fiber as well. The site of action of quinine in muscle has been postulated to be in part, at least, beyond the point of innervation, Oester (1939). Quinine is also known to depress the sensitivity of denervated muscle
to acetylcholine.

We have shown that in the quinine treated rat a tetanus produces a pronounced depression of the muscle response, both mechanical and electrical. This is especially true in the sub-maximal response region. The threshold to the stimulus has in effect been raised.

M. Effect of hormones. It is believed that acetylcholine is essential for the maintenance of optimal neuromuscular function. Alterations in the sensitivity of effector cells to acetylcholine or inhibition of cholinesterase invariably lead to modifications in the responses of the neuromuscular system.

It has been suggested that a decrease in acetylcholine synthesis within the nerve tissue may lead to impaired muscle function. This is demonstrable by a decrease in amplitude during rapid repetitive stimulation in hypophysectomized rats, Torda and Wolff (1950).

A study of the action of the adrenocorticotrophic hormone (ACTH) of the pituitary gland in the myasthenic suggests that this hormone may be a necessary factor in the regulation of neuromuscular function. In this disease ACTH produces a return to normal of the muscle action
potential and recovery of the ability of the blood serum from patients so treated to support acetylcholine synthesis, Torda and Wolff (1951). This drug produced a partial remission which consisted in the patients ability to perform more work while receiving less neostigmine bromide. The following experiments indicate that there is a close relation between the ability of nerve to maintain good function during repetitive stimulation and the ability of nerve tissue to synthesize acetylcholine. Experiments in the rat have shown that after hypophysectomy there is an impairment of neuromuscular function. This dysfunction is evidenced by a decrease in the ability of muscle to maintain the amplitude of the action potential within normal limits during a rapid repetitive indirect stimulation, Torda and Wolff (1952). These authors studied the effect of ACTH in relieving this impaired ability of muscle to maintain its electrical activity. It was found that ACTH administration led to a complete restoration of the neuromuscular response. Cortisone only partly relieved this dysfunction, approximately thirty-three per cent. This work further demonstrated that ACTH increased significantly the ability of brain tissue of hypophysectomized rats to synthesize acetylcholine. Cortisone
acetate administration produced qualitatively similar but quantitatively significantly less effect on acetylcholine synthesis.

Adrenalectomy has also been shown to induce changes in the action potential of muscle contraction, Walker (1948). The increased duration of electrical activity seen in adrenalectomized animals is coexistent with an increase in muscle twitch tension.

The behavior of the muscle action potential during indirect stimulation thus appears to be a resultant of the function of the muscle and nerve. The maintenance of the response of muscle and nerve at a high level appears to be dependent upon optimal endocrine function.

At present the mode of action of hormones and other biological compounds on muscle activity is almost completely obscure. Any effects that may appear are difficult to explain. The metabolism of acetylcholine is a resultant of many factors, such as its synthesis, its hydrolysis, and the sensitivity of effector cells.

The main regulatory mechanisms of optimal acetylcholine metabolism are almost completely unknown. The administration of such compounds as cortisone or ACTH
induce many changes in the blood and tissue of a variety of substances, e.g. electrolytes, metabolites, steroid substances, etc.

The effect of chronic cortisone administration in rats is evidenced in three of our findings, namely, (1) a depressant effect on muscle action potentials during tetanus, (2) a suppression of the normal post-tetanic potentiation of muscle twitch tension, and (3) a post-tetanic depression of action potentials in response to maximal single indirect stimuli.

This leads us to state that chronically administered cortisone may produce a quinine-like effect on muscle. The temporary remission of symptoms in some myotonic patients, treated with cortisone, may have been a result of the above-mentioned quinine-like effects. One would not expect however, that this slight degree of muscle depression produced by cortisone injections would be an effective anti-myotonic agent in all cases.
CHAPTER VI

SUMMARY

The response of the rat gastrocnemius muscle to pre and post-tetanic stimulation, has been determined by the method of graded stimuli. We have established a series of tension-voltage curves for normal rat muscle. These normal responses of muscle have been compared with those obtained during treatment of the experimental animals with d-tubocurarine, prostigmine, quinine, and cortisone. The tension-voltage curves obtained during treatment with these drugs have been discussed.

We have shown that post-tetanic potentiation of the muscle twitch in the normal animal is in some respects different from the non-tetanus induced potentiation obtained with prostigmine. In the normal animal the maximal muscle twitch response is potentiated immediately following a tetanus. The weak and moderate sub-maximal responses are potentiated more slowly.

With prostigmine and in the absence of tetanus the muscle twitch is potentiated in response to all strengths of stimuli from the beginning of the drug effect.

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The tension-voltage curves obtained during progressive curarization demonstrate the marked depression of muscle twitch tension in response to weak and moderate indirect sub-maximal stimuli.

We have also shown that prostigmine and small doses of d-tubocurarine have a similar effect on post-tetanic potentiation in rat muscle. Both of these substances diminish post-tetanic potentiation and may even bring about complete reversal of potentiation, that is, depression.

The marked depression of post-tetanic muscle twitches in response to weak and moderate sub-maximal stimuli which is observed following quinine treatment was also seen in most cortisone treated animals.

We have shown that in normal rat muscle the action potentials of single twitches following a tetanus are reduced in amplitude throughout the entire range of graded response. These action potentials regain normal amplitude in approximately five minutes.

After prostigmine injection the total duration of electrical activity of single muscle twitches is considerably increased, at the same time the spike amplitude is decreased. Following a tetanus the electrical response of the
prostigmine treated rat muscle shows a decrease in duration of the action potential and an increase in the amplitude of the action spike.

We have shown that quinine, d-tubocurarine, and prostigmine have a depressing effect on the action potential of rat muscle during rapid repetitive indirect stimulation.

We have also observed in the chronic cortisone treated rat a quinine-like depression of the muscle action potential during rapid repetitive indirect stimulation.
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## APPENDIX

### TABLE 1

Post-Tetanic Potentiation of Rat Muscle Twitch

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Pre-tetanic twitch response</th>
<th>Muscle twitch response to single stimuli in gastr. muscle of rat following tetanus in (mm)</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/2 min.</td>
<td>3/4 min.</td>
</tr>
<tr>
<td>.30</td>
<td>9</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>.32</td>
<td>11</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>.34</td>
<td>13</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>.36</td>
<td>14</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>.38</td>
<td>16</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>.40</td>
<td>18</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>.42</td>
<td>20</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>.44</td>
<td>20</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>.46</td>
<td>21</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>.48</td>
<td>21</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>.50</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>.60</td>
<td>22</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>.90</td>
<td>22</td>
<td>30</td>
<td>27</td>
</tr>
</tbody>
</table>

Data from this table used in constructing Figure 1
### TABLE II

RESPONSE OF DENERVATED RAT MUSCLE TO DIRECT STIMULATION

<table>
<thead>
<tr>
<th>Volts</th>
<th>1/4 (mm) a</th>
<th>1 (mm)</th>
<th>2 (mm)</th>
<th>5 (mm)</th>
<th>10 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>1</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>40</td>
<td>4</td>
<td>9</td>
<td>15</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>13</td>
<td>18</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>60</td>
<td>11</td>
<td>16</td>
<td>20</td>
<td>25.5</td>
<td>26</td>
</tr>
<tr>
<td>70</td>
<td>13</td>
<td>18</td>
<td>22.5</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>80</td>
<td>15</td>
<td>19</td>
<td>23.5</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>90</td>
<td>16</td>
<td>21</td>
<td>25</td>
<td>31</td>
<td>28.5</td>
</tr>
<tr>
<td>100</td>
<td>17</td>
<td>22</td>
<td>26</td>
<td>32</td>
<td>28.5</td>
</tr>
</tbody>
</table>

-a-mm = twitch height in millimeters

Data from this table used in constructing Figure 2
TABLE III

PROSTIGMINE POTENTIATION OF MUSCLE TWITCH RESPONSE

<table>
<thead>
<tr>
<th>Volts</th>
<th>Pre-Prostigmine injection response (mm)</th>
<th>1 min.</th>
<th>2 min.</th>
<th>3 min.</th>
<th>4 min.</th>
<th>5 min.</th>
<th>10 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.35</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>0.40</td>
<td>14</td>
<td>19</td>
<td>25</td>
<td>30</td>
<td>36</td>
<td>39</td>
<td>49</td>
</tr>
<tr>
<td>0.45</td>
<td>25</td>
<td>34</td>
<td>42</td>
<td>49</td>
<td>55</td>
<td>62</td>
<td>73</td>
</tr>
<tr>
<td>0.50</td>
<td>34</td>
<td>42</td>
<td>52</td>
<td>59</td>
<td>65</td>
<td>72</td>
<td>79</td>
</tr>
<tr>
<td>0.60</td>
<td>34</td>
<td>42</td>
<td>53</td>
<td>59</td>
<td>66</td>
<td>72</td>
<td>79</td>
</tr>
<tr>
<td>0.80</td>
<td>34</td>
<td>43</td>
<td>54</td>
<td>60</td>
<td>67</td>
<td>73</td>
<td>79</td>
</tr>
</tbody>
</table>

To accompany Figure 3
TABLE IV

PROSTIGMINE EFFECT ON RAT MUSCLE TWITCH
AND ON POST-TETANIC RESPONSE

A (see fig. 4A)

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Muscle twitch response to a series of single stimuli prostigmine (0.2 mg/kg i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minutes after injection</td>
</tr>
<tr>
<td></td>
<td>1 min.</td>
</tr>
<tr>
<td>0.35</td>
<td>7</td>
</tr>
<tr>
<td>0.40</td>
<td>16</td>
</tr>
<tr>
<td>0.45</td>
<td>20</td>
</tr>
<tr>
<td>0.50</td>
<td>25</td>
</tr>
<tr>
<td>0.60</td>
<td>32</td>
</tr>
<tr>
<td>0.80</td>
<td>35</td>
</tr>
</tbody>
</table>

(a) Muscle twitch height in mm.
(b) % = post-tetanic twitch height + pre-tetanic twitch height x 100

B (see fig. 4B)

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Muscle twitch response to single stimuli after tetanus in prostigminized rat (Continuation of above experiment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minutes after tetanus</td>
</tr>
<tr>
<td></td>
<td>1 min.</td>
</tr>
<tr>
<td></td>
<td>mm %</td>
</tr>
<tr>
<td>0.35</td>
<td>7</td>
</tr>
<tr>
<td>0.40</td>
<td>16</td>
</tr>
<tr>
<td>0.45</td>
<td>20</td>
</tr>
<tr>
<td>0.50</td>
<td>25</td>
</tr>
<tr>
<td>0.60</td>
<td>32</td>
</tr>
<tr>
<td>0.80</td>
<td>35</td>
</tr>
</tbody>
</table>

To accompany Figure 4.
TABLE V

**EFFECT OF PROSTIGMINE ON THE POST-TETANIC POTENTIATION OF MUSCLE TWITCH IN RAT MUSCLE**

Table A

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Control muscle response in un-treated animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minutes after tetanus</td>
</tr>
<tr>
<td></td>
<td>30 sec.</td>
</tr>
<tr>
<td>1 6</td>
<td>9 150</td>
</tr>
<tr>
<td>2 10</td>
<td>15 150</td>
</tr>
<tr>
<td>3 12</td>
<td>18 150</td>
</tr>
</tbody>
</table>

(a) Muscle twitch height in mm.
(b) \( \% = \) post-tetanic twitch height + pre-tetanic twitch height \( \times 100 \)

Table B

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 sec.</td>
</tr>
<tr>
<td>1 29</td>
<td>13 47</td>
</tr>
<tr>
<td>2 35</td>
<td>19 56</td>
</tr>
<tr>
<td>3 37</td>
<td>20 56</td>
</tr>
</tbody>
</table>

Table C

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 sec.</td>
</tr>
<tr>
<td>1 11</td>
<td>12 110</td>
</tr>
<tr>
<td>2 14</td>
<td>16.5 120</td>
</tr>
<tr>
<td>3 14</td>
<td>18.5 133</td>
</tr>
</tbody>
</table>

To accompany Figure 6
TABLE VI

Alteration in the Response of Normal Rat Muscle to Single Indirect Motor Nerve Stimuli after Injection of d-Tubocurarine (0.20 units/KG - Intraperitoneally)

Muscle twitch response to a series of single motor nerve shocks.

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Pre-injection response</th>
<th>Muscle twitch height at indicated minutes after injection of d-tubocurarine (0.20 u/Kg i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-injection response</td>
<td>20 min.</td>
</tr>
<tr>
<td>0.30</td>
<td>1</td>
<td>1½</td>
</tr>
<tr>
<td>0.35</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>0.40</td>
<td>19</td>
<td>15½</td>
</tr>
<tr>
<td>0.50</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>0.60</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>0.80</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>1.00</td>
<td>31</td>
<td>29</td>
</tr>
</tbody>
</table>

To accompany Figure 8
### TABLE VII

**Effect of d-Tubocurarine on Post-Tetanic Muscle Twitch Potentiation in Rat Muscle**

**Table A**

<table>
<thead>
<tr>
<th>Control muscle response in untreated animal</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Volts (mm)</td>
<td>mm %</td>
</tr>
<tr>
<td>3</td>
<td>23 127</td>
</tr>
<tr>
<td>5</td>
<td>32 128</td>
</tr>
<tr>
<td>8</td>
<td>38 131</td>
</tr>
</tbody>
</table>

(a) Muscle twitch height in mm.
(b) \(\%\) post-tetanic twitch height + pre-tetanic twitch height \(\times 100\)

**Table B**

<table>
<thead>
<tr>
<th>Twenty-five minutes after injection of d-tubocurarine (0.20 units/kgm. intraperitoneally)</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Volts (mm)</td>
<td>mm %</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
</tr>
</tbody>
</table>

**Table C**

<table>
<thead>
<tr>
<th>Sixty-five minutes after injection of d-tubocurarine (0.20 units/kgm. intraperitoneally)</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Volts (mm)</td>
<td>mm %</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
</tr>
</tbody>
</table>

To accompany Figure 9
TABLE VIII

Post-Tetanic Muscle Response Following Quinine Treatment in Rats

Table A

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Pre-tetanic twitch response</th>
<th>Minutes after tetanus (mm)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 min.</td>
<td>2 min.</td>
</tr>
<tr>
<td>.65</td>
<td>34</td>
<td>19</td>
<td>56</td>
</tr>
<tr>
<td>.70</td>
<td>43</td>
<td>38</td>
<td>88</td>
</tr>
<tr>
<td>.90</td>
<td>43</td>
<td>50</td>
<td>116</td>
</tr>
</tbody>
</table>

(a) Muscle twitch height in mm.
(b) % = post-tetanic twitch height / pre-tetanic twitch height x 100

Table B

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Pre-tetanic twitch response</th>
<th>Minutes after tetanus (mm)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 min.</td>
<td>4 min.</td>
</tr>
<tr>
<td>.65</td>
<td>14</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>.70</td>
<td>27</td>
<td>15</td>
<td>56</td>
</tr>
<tr>
<td>.90</td>
<td>37</td>
<td>47</td>
<td>127</td>
</tr>
</tbody>
</table>

Table C

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Pre-tetanic twitch height (mm)</th>
<th>Minutes after tetanus (mm)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 min.</td>
<td>2 min.</td>
</tr>
<tr>
<td>.35</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>.37</td>
<td>18.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>.40</td>
<td>28</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>.50</td>
<td>28</td>
<td>35</td>
<td>125</td>
</tr>
</tbody>
</table>

To accompany Figure 11
### TABLE IX

**POST-TETANIC MUSCLE RESPONSE FOLLOWING CHRONIC CORTISONE TREATMENT IN RATS**

#### Table A

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Pre-tetanic twitch height</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/2 min.</td>
</tr>
<tr>
<td>.40</td>
<td>7</td>
<td>7 100</td>
</tr>
<tr>
<td>.45</td>
<td>25</td>
<td>25 100</td>
</tr>
<tr>
<td>.50</td>
<td>25</td>
<td>31 119</td>
</tr>
</tbody>
</table>

#### Table B

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Pre-tetanic twitch height</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/2 min.</td>
</tr>
<tr>
<td>.60</td>
<td>16</td>
<td>3 19</td>
</tr>
<tr>
<td>.65</td>
<td>26.5</td>
<td>11 42</td>
</tr>
<tr>
<td>.70</td>
<td>37</td>
<td>49 132</td>
</tr>
</tbody>
</table>

#### Table C

<table>
<thead>
<tr>
<th>Volts (mm)</th>
<th>Pre-tetanic twitch height</th>
<th>Minutes after tetanus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/2 min.</td>
</tr>
<tr>
<td>.40</td>
<td>27</td>
<td>5 18</td>
</tr>
<tr>
<td>.45</td>
<td>40</td>
<td>25 62</td>
</tr>
<tr>
<td>.50</td>
<td>45</td>
<td>50 110</td>
</tr>
</tbody>
</table>

To accompany Figure 12
# TABLE X

Alteration in Amplitude of Action Potential Following Tetanus

<table>
<thead>
<tr>
<th>Indirect Stimulus</th>
<th>Pre-Tetanic Spike Amplitude (mv)</th>
<th>Minutes After Standard Tetanus a</th>
<th>1 minute (mv)</th>
<th>3 minutes (mv)</th>
<th>5 minutes (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>.30</td>
<td>35</td>
<td></td>
<td>10</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>.31</td>
<td>65</td>
<td></td>
<td>10</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>.32</td>
<td>90</td>
<td></td>
<td></td>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td>.33</td>
<td>95</td>
<td></td>
<td>35</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>.34</td>
<td>100</td>
<td></td>
<td>40</td>
<td>55</td>
<td>85</td>
</tr>
<tr>
<td>.35</td>
<td>105</td>
<td></td>
<td>50</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td>.36</td>
<td>115</td>
<td></td>
<td>70</td>
<td>70</td>
<td>115</td>
</tr>
<tr>
<td>.37</td>
<td>120</td>
<td></td>
<td>70</td>
<td>75</td>
<td>115</td>
</tr>
<tr>
<td>.38</td>
<td>120</td>
<td></td>
<td>75</td>
<td>80</td>
<td>125</td>
</tr>
<tr>
<td>.39</td>
<td>115</td>
<td></td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>.40</td>
<td>115</td>
<td></td>
<td>75</td>
<td>100</td>
<td>120</td>
</tr>
</tbody>
</table>

a - Standard Tetanus = 100 Sti/Sec at twice Maximal Response Voltage.
b - mv = Amplitude (A) of Action Potential in Millivolts.

Data in this table used in constructing Figure 13.
### TABLE XI

POST-TETANIC ALTERATION IN ELECTRICAL ACTIVITY OF RAT MUSCLE, IN NORMAL, IN QUININE INJECTED, AND IN CORTISONE INJECTED RATS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Per Cent alteration in Amplitude of Action Potential</th>
<th>Per Cent Alteration in Duration of Action Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 min.</td>
<td>3 min.</td>
</tr>
<tr>
<td>Normal rat 1</td>
<td>65</td>
<td>84</td>
</tr>
<tr>
<td>Normal rat 2</td>
<td>68</td>
<td>86</td>
</tr>
<tr>
<td>Normal rat 3</td>
<td>82</td>
<td>86</td>
</tr>
<tr>
<td>Normal Average</td>
<td>72b</td>
<td>86</td>
</tr>
<tr>
<td>Quinine injected rat 1</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Quinine injected rat 2</td>
<td>31</td>
<td>40</td>
</tr>
<tr>
<td>Quinine Average</td>
<td>33</td>
<td>40</td>
</tr>
<tr>
<td>Cortisone injected rat 1</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>Cortisone injected rat 2</td>
<td>16</td>
<td>92</td>
</tr>
<tr>
<td>Cortisone Average</td>
<td>63</td>
<td>88</td>
</tr>
<tr>
<td>Cortisone injected rat 3</td>
<td>68</td>
<td>80</td>
</tr>
<tr>
<td>Cortisone Average</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Cortisone injected rat 4</td>
<td>68</td>
<td>95</td>
</tr>
</tbody>
</table>

**a** - Minutes after Standard Tetanus  
**b** - % of control response (average)

Data from this table used in Figure 14.
### TABLE XII

**EFFECT OF PROSTIGMINE AND TETANUS ON ACTION POTENTIALS OF RAT MUSCLE TWITCHES**

<table>
<thead>
<tr>
<th>Minutes after injection of prostigmine</th>
<th>Amplitude of action potential (A) (mm)</th>
<th>Total duration of action potential (RT) (mm)</th>
<th>Duration of first diphasic response (RS) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>1½</td>
<td>14</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>2½</td>
<td>10</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>3½</td>
<td>9</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>4½</td>
<td>9</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>12</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minutes after 30 second tetanus</th>
<th>Amplitude of action potential (mm)</th>
<th>Total duration of action potential (mm)</th>
<th>Duration of first diphasic response (RS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1½</td>
<td>11</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1¾</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2½</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>3½</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4½</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Data in this table used in constructing Figure 16
APPROVAL SHEET

The dissertation submitted by John F. Polli has been read and approved by five members of the faculty of the Stritch School of Medicine.

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 dissertation 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.

2290m53
Date

Y.T. Ortner
Signature of Adviser