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## Tetanus Toxin and Skeletal Muscle Electromyographic, Electrodiagnostic and Pharmacological Study

Venkatray G. Prabhu  
*Loyola University Chicago*

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TETANUS TOXIN AND SKELETAL MUSCLE  
ELECTROMYOGRAPHIC, ELECTRODIAGNOSTIC  
AND PHARMACOLOGICAL STUDY

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by  
Venkatray G. Prabhu

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

February

1962

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## APPROVAL SHEET

The dissertation submitted by Venkatray G. Prabhu has been read and approved by five members of the faculty of the Graduate School.

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

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

23 Jan 1962  
Date

Y. T. Oster  
Signature of Advisor

## LIFE

Venkatray Govind Prabhu was born in Shirali, Bombay State (INDIA), in March, 1930.

He attended the University of Bombay, receiving a B.Sc. in chemistry in 1953, a B.Sc. (Tech) in pharmaceuticals and fine chemicals in 1955 and a M.Sc. (Tech) in pharmacology in 1958.

Since 1959 he had been a graduate student in the Department of Pharmacology, Stritch School of Medicine, Loyola University. He was a graduate assistant from 1959-61.

He is a member of the Indian Pharmaceutical Association and was its Hon. Secretary (1955-57), and a member of the Technological Association, Bombay.

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

- 1) Prabhu, V.G., 1955. Pharmaceutical Industry in India. Bombay Technologist, V: 47-49.
- 2) Prabhu, V.G., 1956. Salk Vaccine. Bombay Technologist, VI: 32-35.
- 3) Prabhu, V.G., 1957. Ion Exchange in Industry and Medicine. Bombay Technologist, VII: 1-3.
- 4) Khorana, M.L., V.G. Prabhu and M.R. Rajarama Rao, 1958. Pharmacology of an Alcoholic Extract of Plantago Ovata. Indian Journal of Pharmacy, 20: 3-6.
- 5) Prabhu, V.G., Y.T. Oester and J.J. Fudema, 1961. Tetanus Toxin and Skeletal Muscle. Fed. Proc., 20: 306.
- 6) Proctor, C.D., S.A. Ridlon, J.J. Fudema and V.G. Prabhu, 1961. Factors Affecting the Extension of Hexobarbital Effect by Anticholinesterases. Fed. Proc., 20: 395.



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## CHAPTER I

### INTRODUCTION

Claude Bernard (1875) in his book entitled "Experimental Science" began the account of the action of curare with the following remarks: "Poisons can be employed as means for the destruction of life or as agents for the treatment of the sick, but in addition to these two well-recognized uses there is a third of particular interest to the physiologist. For him, the poison becomes an instrument which dissociates and analyses the most delicate phenomenon of living structure and by attending carefully to their mechanism in causing death, he can learn indirectly much about the physiological processes of life. Such is the way in which I have long regarded the actions of toxic substances". One such toxic substance, useful for the pharmacologist for the purposes envisioned by Claude Bernard is tetanus toxin. This is a toxin formed by the bacterium *Clostridium tetani*.

Tetanus toxin has been a subject of vigorous experimental study for the last 80 years. It is one of the most potent bacterial neurotoxins ever studied. It is lethal in such minute dosages, in almost all animal species, that it has drawn the attention of bacterial toxicologists and physiologists.

The infectious nature of the tetanus organism was first incontrovertably demonstrated by Carle and Rattone in 1884 when

they succeeded in inducing the disease experimentally in rabbits by inoculation with finely divided tissue from a patient who had died of tetanus. All rabbits except one died after reproducing the classical signs of the disease. The causative organism was identified later as a bacillus and known as *Clostridium tetani* (Nicholair, 1884). From the culture of this anaerobic, spore bearing bacillus, a highly lethal toxin was obtained by filtration, which on inoculation produced the typical features of naturally occurring tetanus in man and animals (Tizzoni and Cattani, 1890a, 1890b, and Faber, 1890).

Clinically tetanus falls under two general catagories: one, the "general" in which after an ill defined first stage, the intoxication manifests itself by an increasing spasticity of the muscles of mastication followed by similar changes in the muscles of trunk and limb, and still later by generalized convulsions; and second, the "local" in which the local region of infection becomes at first painful and later spastic. Experimentally both these forms can be readily reproduced in labroatory animals - the "general" by intravenous and the "local" by intramuscular injection of the toxin.

In the naturally occurring tetanus the bacilli usually enter the tissue in the form of spores through a cut or wound. Once in the tissue, the spores undergo metamorphosis. The appropriate nutritive medium, the pH and the temperature of the lacerated tissue are highly conducive to the germination of the



spores. During the multiplication process, toxin is formed at the infected foci in the tissue. At the time of death, large deposits of toxin are found to be present around the growing bacteria (Francis, 1914). One report, available in the literature, of an outbreak of tetanus in St. Louis comprised seven children who were given diphtheria antitoxin. This had been inadvertently obtained from a horse, two days before it developed clinical signs of tetanus. Abel and his colleagues (1934) calculated that at this stage of incubation of the disease, the animal had 280 horse lethal doses of toxin.

#### Pathogenicity of the Toxin

Fatal intoxication can be induced in a wide variety of both warm and cold blooded animals. However, the minimum dosages of the toxin required varies from one species to another. This, further, depends upon a variety of factors such as the strain of clostridium used, method of preparation of toxin, the breeds, sexes and the ages of the animals employed, as well as many other experimental conditions. In general, it can be concluded that a large group of animals consisting of horse, monkey, sheep, goat, guinea pig, and mouse is highly susceptible. Cats and dogs are less vulnerable, while birds are strikingly resistant. From accidental inoculations, it appears that man is among the more susceptible species (Nicholas, 1893; Bolton and Disch, 1902). It has been said that the rabbit is the most unpredictable animal. Von Behring (1912) was the first to comment on this finding on

rabbits. He suggested that the blood vessel endothelium of rabbits possesses a destructive enzyme to which he gave the name tetanotoxinase. No further work along this line appears to have been undertaken. Work by Lamont and his associates (1940) and Ipsen (1940-41) on different varieties of rabbits, with the same toxin and with toxins from different strains of *Cl. tetani*, indicate that the variations in the results with rabbits lie in the differences in the "biological qualities" of the toxin, rather than in the species variability of rabbits.

#### Route of Infection and Lethality of the Tetanus Toxin

The route of infection of the toxin into the body not only determines many of the clinical manifestations but also the size of the requisite lethal dose. The closer the site of inoculation approaches the vital centres in the brain stem, the more effectively lethal does this toxin become, irrespective of the species difference.

#### Site of Action of Tetanus Toxin

The students of tetanus all agree that in "general" tetanus, in which spasticity is widespread in skeletal musculature and convulsions occur frequently, the toxin operates at some site within the central nervous system. With "local" tetanus, however, there still remains a sharp difference of opinion as to whether all the manifestations can be wholly accounted for by an action on segments of the central nervous system, or whether the toxin

brings about some of degree of local spasticity through a peripheral action on susceptible structure in the affected muscles themselves. This question has not been solved satisfactorily. Most observers have inclined to the view that in all forms of tetanus, the toxin operates only on the elements of the central nervous system. The disagreement appears to be due to lack of appreciation by some investigators that affected skeletal musculature passes through a succession of clinical stages as local tetanus progresses. This was properly emphasized by Ranson and his associates (1926, 1928, 1929). They described two characteristic forms of spasticity, an early one lasting roughly 5-6 days, in which the contraction of the affected muscle can be terminated by general anesthesia, curariform drugs or nerve section, and a later one, in which the muscle remains firmly contracted for many weeks in spite of isolation from the central nervous system by denervation or by drug action. They called the former stage "hypertonic contraction" and the latter "myostatic contracture".

#### A Peripheral Site of Intoxication

Vaillard and Vincent (1891) showed that: for the clinical manifestations of local tetanus the intact motor innervation is necessary because local tetanus could not be produced after sectioning of the nerve to the local muscle. Abel and his associates (1935a,b,c, and 1938) held the view that the spasticity could originate from some toxic injury to the peripheral ending of the motor neurons and that it is overactivity at the motor end

plate that maintained the unremitting contracture. Harvey (1939) recorded some observations along this line: (a) the spasticity persists in the intoxicated muscle several days after the sectioning of the motor nerve and disappears with the degeneration of nerve ending; (b) the spasticity can develop in the denervated limb if the toxin is injected directly into the muscle before the motor endings lose their transmitting function; (c) a single nerve volley produced by electrical stimulation of a motor nerve trunk- which usually gives a single action potential in normal muscle - leads to a repetitive discharge in the intoxicated muscle.

Harvey's observations have been severely criticized by a large number of later investigators. One of the criticisms is related to Harvey's denervation technique. While he sectioned the nerve in the thigh other investigators had observed that for complete denervation of the hind limb, sectioning of femoral and obturator nerve is necessary, Courmont and Doyon (1899), Permin (1913), Pochhammer (1909), Sawamura (1909). Further, Permin had pointed out that in rabbits there is an additional trunk in the sacral plexus which may escape division. By taking necessary precautions to ensure complete denervation Hutter (1951) showed that no signs of local tetanus were produced. Harvey's observations on the electromyograms of tetanus intoxicated muscle, in which repetitive action potentials followed a single nerve stimulus, have been criticized by several later workers who attempted to repeat his work. Gopfert and Schaefer (1940), Acheson, Ratnoff

and Schoenbach (1942), Purdrup (1946), Mackereth and Scott (1954) have all been unable to confirm his finding. Gepfert and Schaefer suggested that Harvey's recorded action potentials were artefacts possibly caused by the spread of electrotonus from the stimulating electrodes onto the nerve end-plates in the muscle.

### Proprioceptive Sensory Nerve Endings

Convulsive motor activity in local tetanus can be induced and exaggerated by reflex stimulation of proprioceptive and other sensory end organs. Early workers attempted to determine the extent to which the spasticity in local tetanus can be dependent on the afferent nerves. Results on de-afferented animals (by the division of posterior nerve roots) are not unanimous. Thus, Courmont and Doyon (1899) prevented local tetanus by this method. Gumprecht (1894) with dogs, Brunner (1894) and Goldschieder (1894) with rabbits and Frohlich and Meyer (1915), Liljestrand and Magnus (1919) and Ransom (1928) with cats reported that posterior root section might diminish but not abolish local tetanus. Whatever may be the inconclusive nature of the early work on de-afferentation, the excessive activity, during the early "hypertonic contraction" stage of local tetanus is certainly enhanced by the afferent impulses reaching the spinal cord.

Schaefer (1944) supported this statement by two kinds of experimental observations (a) an increase in the number of action potentials recorded from the afferent nerve of tetanus intoxicated muscle. This augmentation in the number of action potentials was

regarded by him as an indication of a pathological sensitivity of the intoxicated muscle, (b) an increase in the activity in the control limb when tension was applied on the tetanus side and recording was made from the control side. Another approach to the problem was made through the use of a local anesthetic, procaine, which, in the concentration which was used, is believed to anesthetize only the sensory receptors without paralyzing the myoneural junction. Liljestrand (1919), Ranson (1928) and Purdrup (1946) used this method and found that the injection of dilute solution of procaine into a spastic muscle abolished the characteristic activity without affecting the normal voluntary or reflex function. These findings, however, do not prove the site of action of tetanus toxin. Friedemann and his colleagues (1941) repeated some of the earlier work in which posterior roots had been sectioned before the injection of tetanus toxin. They used monkeys with de-afferented muscles and found that all the animals developed local tetanus on the third or fourth day. Davis, Morgan and Payling Wright (1954) used electromyographic methods to study local tetanus in rabbits, and found that during the early stages of hypertonic contraction the spasticity and the "shower" like electromyographic activity can be promptly suppressed by i.v. injection of a moderate dose of pentobarbital. This dose of pentobarbital however, did not alter the patellar tendon reflexes, either in the normal or intoxicated limb. Moreover, the electromyographic record of the tendon jerk possessed the same shape and

amplitude after the spasticity had been abolished by pentobarbital as it had before. This indicated, therefore, that the monosynaptic reflex arc was unaffected by tetanus toxin and the spontaneous spinal cord discharges down the motor nerve trunk had their origin at another site.

#### Central Site of Intoxication

Some of the earliest and most convincing evidence was brought forward by Meyer and Ransom (1903) when they found that typical tetanus can be induced by the inoculation of very small amounts of toxin intraneurally into the sciatic nerve trunk or the spinal cord. In both cases typical tetanus developed in the muscles of the hind limb. These experiments have been repeated during the past half century with the same results - Baylis et al (1952), Horster et al (1931-32), Permin (1913-14), Sherrington (1906) and Teale and Embleton (1920). Abel, Hampil and Jonas (1935) were the only investigators who questioned this method of producing local tetanus. The major objection of Abel's group was with the technical non-feasibility of making such intraneural injections without retrograde leakage of some of the toxin along the track of the needle when it is withdrawn. However, other evidences in the literature greatly diminished Abel's argument.

Davis, Morgan and Payling Wright (1954) showed that when small quantities of tetanus toxin are injected into the sciatic or femoral nerve trunks of rabbits in the vicinity of the lumbar and sciatic plexuses, individual electromyograms taken from the main

muscle groups in the limb show two distinct patterns of local tetanus. With femoral nerves the quadriceps extensors of the thigh alone are involved. With the sciatic nerve, not only are the hamstring muscles of the thigh affected, but typical tetanus also appears at the same time, in both the tibialis anticus and the calf group of muscles. This situation is compatible with the anatomical distribution of nerve and muscles in the hind limbs of cats and probably rabbits as pointed out by Ramones (1951). Similar evidence for a central site of intoxication comes from the observation of Mackereth and Scott (1954) that local tetanus develops in the diaphragm after the inoculation of muscles which are anatomically remote from it but which possess a common segmental innervation.

Wright, Morgan and Payling Wright (1950) inoculated toxin solutions into each of the 3 main cranial motor nerves which are more complex in their muscular distribution than the lumbar nerves. When the toxin was injected into facial, vagus or hypoglossal nerve trunks, the same typical forms of bulbar tetanus - viz. the strabismus, torticollis, salivation, bradycardia etc. - were obtained. It is inconceivable to assume that all these diversified effects could occur by chance contamination of neighboring areas during injection. Further since the whole syndrome of bulbar tetanus is the same irrespective of whether the toxin is injected into vagus, facial or hypoglossal nerve, the simplest explanation, according to the authors, was that all these signs



resulted from the central action of the toxin on the closely spaced brain stem nuclei, whose neurons provide the final common pathway for the activation of these diverse effector organs.

Further evidence for the initial central rather than peripheral action of tetanus toxin was produced by Wright, Morgan and Payling Wright (1950). These investigators temporarily blocked the hind limbs of rabbits from the general circulation by inflating a suitably placed sphigmomanometer cuff. In local tetanus closure of the limb arteries for a short time has no effect on the electromyographic pattern. But when pentobarbital was injected into the general circulation at the time when its access to the leg is prevented by high vascular occlusion, all traces of both spasticity and the "showering" of electromyographic activity disappeared completely within fifteen seconds.

Since the symptoms of local tetanus were abolished even when the entry of pentobarbital into the affected limb was blocked, the conclusion was that the spasticity and the electromyographic pattern of local tetanus was mediated through the centrally situated neural structures to which the anesthetic had unimpeded access. This finding is irreconcilable with Harvey's belief that the spasticity arises from a peripheral intoxication at the myoneural junction.

#### Spread of Tetanus Toxin From a Depot Site in the Tissues

There is a prolonged controversy in the literature as to the possible routes by which this substance can reach the central

nervous system. Two clinical forms of tetanus - the "ascending or local" and "descending or general", produced experimentally by intramuscular and intravenous injections respectively, of tetanus toxin, have been mentioned earlier. There is an overwhelming mass of evidence to prove that in "ascending" tetanus the toxin is carried centripetally along the motor nerve trunks from the depot in the tissue to the spinal cord and brain stem (Baylis et al, 1952, Pochhammer, 1909, Sawamura, 1909). Among the many experiments undertaken to throw light on the pathogenesis of "ascending" tetanus, those designed to produce some obstacle to the centripetal movement of the toxin along the large nerve trunks have been decisive. The three kinds of obstacles that have been set up to lessen or prevent such movements are (a) immunological barrier created by a proximal injection of a specific antitoxin (b) transection of the nerve trunk at some site between the point of injection and the spinal cord (c) structural disorganization of the tissue space in the nerve trunk by intraneuronal injection of some sclerosing agent, several weeks before the injection of toxin.

Meyer and Ransom (1903) first showed that "ascending" tetanus can be prevented by simultaneous injection of specific antitoxin into the regional nerve trunk at some proximal site. This observation has been confirmed by Permin (1913), Sawamura (1909) and Teale and Embleton (1920). This does not, however, exclude the possibility of both toxin and antitoxin moving upwards along the nerve to enter the tissue fluid of the spinal cord and

exerting its specific protecting power there. This possibility could be excluded by creating a zone of sclerosis in the substance of the nerve trunk itself. Teale and Embleton (1920) sclerosed the sciatic nerve trunk of rabbits by injecting tincture of iodine and prevented the appearance of local tetanus by injecting tetanus toxin directly into the muscle of the hind limb. Abel's group correctly criticized this process as being equivalent to "nerve section" by the alcohol present in the tincture. More recently, Baylis et al (1952) Wright, Morgan and Payling Wright (1951) repeated these experiments with milder sclerosing agents such as ethanolamine oleate and prevented the appearance of local tetanus when the toxin was injected below the region of ethanolamine injection. However, injection of toxin proximal to the site of sclerosis did produce local tetanus in the hind limb. Other evidence of this centripetal movement of toxin in the "ascending" tetanus is presented by the work of DeAntona (1949, 1951), Friedemann, Hollander and Tarlow (1941).

Once the centripetal movement of the toxin along the motor nerve trunk is accepted there still remains the uncertainty as to which component of the nerve trunk forms the necessary conduit for its passage. Diffusion alone would be incapable of accounting for the ascent of a protein at such a high rate. Bromeis (1938, 1939), Meyer and Ransom (1903) and others held that tetanus toxin passes upwards in the interior of the motor axons themselves. This, however, is no longer tenable in the light of

microdissection studies of DeRenyi (1929, 1932). One possible mode of the toxin movement is along the highly oriented tubular clefts that lie between the individual nerve fibers and that the force needed for its propulsion is provided by the great increase in pressure of tissue fluid in the muscle belly when it undergoes contraction, Barcroft and Dornhorst, (1949) Baylis et al (1952) Wells et al (1938). This is in agreement with the prevention of the ascent of the toxin by sclerosis of the nerve trunk, Wright (1951).

#### Descending Tetanus

Clinical features of "descending" tetanus, which resembles closely the natural disease in human and animals, can be produced by intravenous injection of toxin into laboratory animals. This similarity suggests that "descending" tetanus results from carriage of toxin to the nervous elements by circulating blood. This supposition is borne out by (a) identification of toxin in the blood (b) comparative ease with which the effect of this toxin can be neutralized with specific antitoxin. The routes taken by the toxin in its passage from blood to susceptible elements in the CNS are still obscure and seem to be closely connected with the physiological and pathological behavior of the postulated blood brain barrier (Friedemann, 1942, 1947). It has been reported (Wislocki, 1920) that certain sites, particularly the areas postremae in the floor of the fourth ventricle permit the entry of substances from blood into nervous elements even though the

capillaries of the CNS are generally known to be impermeable to certain plasma contents, (Krogh, 1946). This local capillary permeability in the floor of the fourth ventricle may be one of the causes responsible for the early signs of tetanus intoxication.

### Mode of Action of Tetanus Toxin

The exact nature of the injury inflicted by tetanus toxin on neural elements is not known. More than 100 years ago Sir James Simptom, while reviewing a long series of cases of peripheral tetanus observed the similarity between this disease and that of strychnine poisoning. Sherrington (1906) was the first to suggest that the common element in the modes of action of the two poisons was their conversion of synaptic inhibition to one of excitation.

Further evidence on the mode of action of tetanus toxin came from the work of Eccles and his collaborators, Bradley, Easton and Eccles (1953). Brooks, Curtis and Eccles (1955) produced experimental evidence for the similarity between the actions of tetanus toxin and strychnine. This was also recognized by Davis, Morgan and Wright (1954). They showed that both tetanus toxin and strychnine, in moderate doses had insignificant action on monosynaptic reflexes. Bradley, Easton and Eccles (1953), Eccles, Fatt and Koketsu (1954) showed that strychnine acts specifically by diminishing all types of synaptic inhibitory actions. Brooks, Curtis and Eccles (1955) employed single maximum volleys along the large stretch afferents as inhibiting volleys on monosynaptic

reflex discharges and constructed inhibitory curves. The injection of tetanus toxin completely abolished this inhibitory influence on the motoneuron, while the monosynaptic reflex spike showed insignificant change on the ipsilateral side and remained virtually the same on both sides. They also showed that tetanus toxin did not exert any effect on the synaptic relays that occur on direct inhibitory pathways, (Eccles, Fatt and Koketsu, 1954, Eccles Fatt and Landgren, 1956).

Intracellular recordings from motoneurons, indicated that inhibitory hyperpolarization is suppressed by tetanus toxin exactly as occurs with strychnine. This was explained by the following two possible mechanisms: (a) that tetanus toxin may prevent the release of transmitter substance in a manner similar to that of botulinum toxin, Brooks, (1956), Burgeon, Dickens and Latimer (1948) (b) like strychnine, tetanus may become attached to the subsynaptic inhibitory areas on the motoneuronal membrane, thus preventing the action of the inhibitory transmitter, (Brooks Curtis and Eccles, 1957).

In order to study the relationship of the various pathways of inhibition entering the spinal cord, Curtis (1959) employed tetanus toxin and strychnine. He concluded that all forms of inhibition (Bradley Easton and Eccles, 1953, Eccles Fatt and Koketsu (1954), Brooks, Curtis and Eccles, 1957, Kuno, 1957) of the motoneurons located within the lumbar segments of the cat, are produced by the same inhibitory transmitter substance. Further,

these inhibitions can be prevented by the action of either strychnine or tetanus toxin. In the cases of both direct inhibition of motoneurons and that of "Renshaw" cells the specific inhibition of the interneuron was not affected by either of these agents. Hence, it was considered these agents may prevent either the release of inhibitory substance or its post-synaptic action. Recently Wilson et al (1960a) showed that injection of tetanus toxin into the spinal cord results in an increase in the polysynaptic reflexes, an appearance of polysynaptic reflexes in some nerves from which they were previously absent, an increase in duration of reflex discharge and a lack of development of spontaneous activity. Tetanus toxin also resembles strychnine in depressing recurrent facilitation and inhibition (Wilson et al 1960).

### Biochemical Changes

Information about the biochemical changes occurring during tetanus intoxication is scanty. Harvey (1939) suggested that tetanus toxin produced a disturbance in the acetylcholine metabolism at the skeletal neuromuscular junction and also a reduction in the cholinesterase activity.

Martini, Torda and Zironi (1939) however, failed to support Harvey's suggestion by their chemical analysis of the gastrocnemius and tibial muscles of rats. Ambachi, Morgan and Wright (1948) also, could not find any appreciable reduction either in the true or pseudo cholinesterase activity in the iris of rabbits, or in the quadriceps femoris and rectus abdominis

muscle of guinea pigs after tetanus toxin treatment. Schaefer (1944) however, observed a slight reduction in the cholinesterase activity in muscle that had been incubated with tetanus toxin for several days. Similar findings have been recorded by Werle and Stuttgart (1942) and by Ammon (1943).

### Electrodiagnosis

Electrodiagnosis is the interpretation of responses of nerves and muscles to electrical stimulation. Electrodiagnostic procedures have long been used in clinics and in experimental laboratories. The electrical constants usually determined are rheobase, strength-duration curve, chronaxie, galvanic-tetanus ratio, etc.

#### Rheobase

Lapicque (1909) developed the concept of rheobase, which means the threshold of excitation. He observed that a minimum current is necessary before an excitation can occur. In other words, no current less than rheobase can produce excitation even if applied indefinitely. Consideration of rheobase alone as a diagnostic guide is questionable. Laroquette (1920) Pollock and his associates (1945) reported that rheobase decreases with progressive nerve degeneration and rises as the reinnervation ensues. Ritchie (1954) failed to observe this. Wynn-Parry (1953) reported variable results. Fudema, Oester, Fizzell and Gatz (1960) confirmed the observation of Pollack's group. Harris (1952) pointed



out such technical factors as electrode size and position, local condition of the tissue, skin temperature, blood supply, edema, etc. are likely to influence the rheobase value.

### Galvanic tetanus ratio

Skeletal muscle contracts with a sharp twitch when a current of rheobase value is applied. When the strength of the current is increased the twitch is transformed into a persistent contraction called "tetanus". Early investigators termed this tetanus state as "Galvanotetanus". Erb (1868) and Mendelsohn (1909) noted that the value of this galvanotetanus was increased in denervated muscles. Pollock et al (1945) proposed a term "Galvanic Tetanus" since it results from galvanic stimulation. Since the absolute value of either Rheobase or Galvanic Tetanus varies with changes in the experimental conditions, the state of the tissue, etc., the ratio of galvanic tetanus and rheobase was considered to be more reliable and Pollock coined the term "Galvanic Tetanus Ratio" for this value. Pollock who observed a large variation in the galvanic tetanus ratio in denervated animals cautions that the interpretation of this data must be considered with reference to the time after denervation. Recently Fudema, Oester, Fizzel and Gatz (1960) have reported an increase in galvanic tetanus ratio in the denervated muscles of rabbits.

### Strength-duration curves

The response of an excitable tissue, such as nerve or

muscle depends upon certain parameters including strength and duration of the applied stimulus. The curve relating these two parameters is known as strength duration curve. Cluzet (1903) was probably the first to graphically represent the excitability characteristics of nerve and muscle using these parameters. Lucas (1906, 1907-08) observed that using non-polarisable electrodes and stimulating the sartorius muscle of frogs with galvanic current, the strength duration curves obtained were complexes consisting of two or three distinct segments. He attributed these segments to the response of (a) muscle fibres to the currents of long duration, (b) nerve fibres to current of short duration, (c) myoneural junctions to the current of very short duration. Adrian (1916) used Strength-Duration curves in patients with lower motor neuron lesions. In progressive studies, on patients with polyneuritis and peripheral nerve injuries, he demonstrated discontinuities during the recovery stages. He also described double curves of partial innervation. Adrian thought that these responses were due to a group of muscle fibres at different stages of recovery. It was not until 1930 that Rushton described in frog muscle the alpha and gamma excitabilities suggested 20 years earlier by Lucas (1907). Later, in 1932 Rushton showed that the alpha component was isochronous with muscle and corresponded to the right-hand portion of the double curve, while the gamma component was isochronous with nerve and corresponded to the left hand portion.

Pollock (1945), Newman and Livingstone (1947), Mackenzie

(1949) and Wynn-Parry (1952) have described the detailed changes in the curves during degeneration and regeneration of the lower motor neuron. Pollock and his coworkers found that in the experimental animal during the period of nerve degeneration following nerve section, before the muscle is completely denervated, discontinuities may occur. Further, the discontinuities disappear and the Strength-Duration curves become continuous when the muscle is completely denervated. Fudema et al (1960) have reported that similar discontinuities also appear in the Strength-Duration curves in vitamin E deficient rabbits.

### Chronaxie

Lapicque (1909) defined and employed the word "chronaxie" to denote the minimal value of the current duration at double the rheobase intensity needed for excitation. This was the first expression embodying both time and intensity. Lapicque mathematically showed that the stimulus of twice rheobase strength with a duration equal to chronaxie, is of all the possible threshold stimuli, the stimulus of least energy. He showed that in normal nerve-muscle studies the Strength-Duration curve was approximately a rectangular hyperbola. This was confirmed by Sokamoto (1933) on single nerve fibres. Lapicque (1926), LaSalle (1926). Bourguignon (1923) considered that chronaxie in itself is an exact index of tissue excitability. However, this view is not generally accepted, Davies (1936) and Rushton (1935).

Lapicque (1909) found that the chronaxie of different

normal muscles in humans varied from 0.2 - 0.5 milliseconds. Following a study of many hundreds of strength-duration curves Ritchie (1954) found the chronaxie to be remarkably constant and that the greatest deviation in observations made on a single muscle of one subject was of the order of 0.02 milliseconds (Voltage stabilized stimulator). The ranges of values he quotes for voltage stabilized and current stabilized stimulators are respectively .03 to .08 and 0.15 to 0.8 milliseconds. Ninety percent of all the observations made on healthy muscles fell within this range. The chronaxie value determined by some investigators on normal muscle (Lucas, 1907-08, Adrian, 1916, Davis, 1922-23) and on denervated muscle (Watts, 1924-25, Pollock et al, 1945 and Fudema et al 1960) may be summarized as follows: in normal muscle the chronaxie is very short 1.0 millisecond or less, and in a completely denervated state the chronaxie is very high, usually above 10 and up to 100 milliseconds.

### Electromyography

Electromyography may be defined as the recording and study of intrinsic electrical activity or production of skeletal muscle. Normal and denervated muscles have been subjected to an intensive electromyographic study, both qualitative and quantitative, since the differences in electromyographic patterns of normal and denervated muscle can be distinctly recognized (Denny-Brown and Pennybacker, 1938, Jasper and Ballem, 1939, Buchthal and Clemessen, 1941, Weddel et al 1944, Golseth and Fizzel, 1947,

Richardson, 1954, Rodriguez, Oester and Skolnik, 1954 and others).

In a normal skeletal muscle at rest an undisturbed isoelectric base line, usually referred to as "electrical silence" is a normal finding. However, in a muscle whose motor nerve has been sectioned and allowed to degenerate (Wallerian degeneration) spontaneous electrical activity is the invariable finding. It is believed that the spontaneous fibrillation potentials represent the electrical activity originating in single muscle fibres.

Normal neuromuscular function is represented electromyographically by the production of electrical activity potentials known as "motor unit potentials". The term motor unit was first introduced by Liddell and Sherrington (1925). A motor unit is defined as the motor nerve cell, its axon, end plates, and all the muscle fibres it innervates. The axon of a motor neuron divides many times to supply many muscle fibers. Thus, an impulse propagated along a motor neuron usually causes all the muscle fibers innervated by the neuron to contract practically simultaneously and as a unit. Consequently, all normal activity of normal skeletal muscle is based upon the integrity and organization of motor units. Immediately following the motor nerve section no motor unit may be initiated voluntarily or reflexly by the subject. In specific myopathies, alteration in the wave form, voltage, sound and other parameters of the electromyogram yields information as to the particular type of pathology, myosthenia gravis, Botello, Deaterly and Comroe (1952) muscular dystrophy, Buchthal and

Pinnelli (1952), myotonia, Adams, Denny-Brown and Pearson (1954), polymyositis, Hines et al (1944).

### Statement of the Problem

Perusal of the related literature on local tetanus indicates that there is a long standing controversy between the proponents of a peripheral and the advocates of the central action of tetanus toxin.

Opinion is unanimous regarding the necessity of the central nervous system for the manifestations of classical symptoms of local tetanus, since this has been unequivocally shown by many investigators.

One question still remains to be settled as to whether or not tetanus toxin, besides its repeatedly emphasized initial CNS effects, also exerts a peripheral effect of any kind. In general, the advocates of the central action of tetanus toxin conducted their experiments mainly during the early stages of local tetanus and hence were convinced that the action of tetanus toxin is only on the central nervous system. A careful survey of the experimental protocols of Abel's group, particularly those of Harvey, suggests that besides the initial central action, tetanus toxin also exerts a peripheral action. The latter is masked by the predominant central action especially in the early stages.

It is the opinion of the author that the discrepancy in the results mentioned above, lies in the failure of the proponents of the central action to take into account the possible delayed

or late peripheral action of the tetanus toxin.

Taking into consideration both possibilities, a systematic study of both early and delayed effects of tetanus toxin in the rabbit hind limb muscle therefore is pursued in this report.

In a preliminary survey, using an electromyographic procedure, it was found that during the early stages of local tetanus (32-48 hours after the injection of toxin) there was a tremendous "shower" of motor unit potentials, which could be reduced in number by light anesthesia or could be enhanced by any kind of reflex stimulus. This obviously is a central effect of tetanus toxin. This stage lasted for about 4-6 days. After about 10 days following the injection of toxin an entirely different phenomena was observed. The motor unit activity disappeared completely and in its place fibrillation potentials were seen. It is well known that fibrillation potentials and increased response to pharmacological agents like Ach, Decamethonium etc. are signs of chronic denervation. The induced local tetanus, in its advanced stages, produced, in the involved muscle, an effect pharmacologically and electrophysiologically analogous to that produced by chronic denervation.

Hence, a systematic electrophysiological and pharmacological investigation on a large number of rabbits in local tetanus was undertaken. Comparable results were also obtained from a series of denervated rabbits. The data will be discussed in the light of current neuromuscular physiological and pharmacological knowledge.

## CHAPTER II

### MATERIALS AND METHODS

#### Experimental Animal

Throughout the work male albino rabbits weighing 1.5-3 kg. were used. Rabbits were maintained on regular "Purina Rabbit Chow" ration supplemented daily with fresh lettuce.

#### Tetanus Toxin

The tetanus toxin used in this work was kindly supplied by Eli-Lilly and Company (#735677, Meuller Medium, containing 1:10,000 Merthiolate). The potency of the toxin as noted on the label, was 800,000 guinea pig MLD/ml. The original toxin was always preserved at refrigerator temperature. Preliminary experiments on rabbits indicated that the following dilution of the toxin from the original, was adequate for the purpose of producing uniform local tetanus. Thus 0.1 ml of the original toxin (800,000 guinea pig MLD/ml) was diluted to 40 ml with 0.90% saline and 0.1 ml/kg (200 guinea pig MLD/kg) of the diluted solution was used for injection.

#### Local tetanus by intramuscular injection of the toxin

The methods used for producing local tetanus were essentially the same as those used by Abel et al (1934), Harvey (1939), Payling Wright and his co-workers (1954). Two hundred guinea pig MLD/kg of the toxin was injected into the right



tibialis anticus muscle by using a #27 gauge needle. In some rabbits the injection was made along the muscle at ten different points. Typical signs of local tetanus appeared 36-48 hours following the injection. There was no apparent difference in the clinical appearance of the rabbit whether the injection was made at one point or at several points in the muscle.

#### Local tetanus by intraneural injection

For this purpose the rabbits were anesthetized with ether. The sciatic nerve was exposed high up in the thigh. By means of a #27 gauge needle bent at 45° angle, 0.05 ml of the toxin solution (200 guinea pig MLD/ml) was injected into the sciatic nerve. The injection was made with the needle pointing towards the spinal cord (Payling Wright, 1952). Care was taken not to contaminate the adjacent muscles with the toxin, while injecting or immediately after the withdrawal of the needle. Contralateral limbs always served as controls.

#### Chronic denervation

The right tibialis anticus muscle was denervated in all the cases. The common peroneal nerve was exposed under ether and a section of the nerve, about 2 cms in length was excised. The incision was closed by Nickel-silver wound clips. No infection was observed in any of these operated animals.

#### Nerve-muscle preparation

Acute nerve-muscle experiments were done as follows:

The spinal cord of the rabbits was sectioned between T<sub>6</sub>-T<sub>12</sub> under ether anesthesia. The tendon of the tibialis muscle was freed and connected to a force transducer to obtain an isometric myogram. A steel pin was driven through the distal head of the femur and fixed rigidly in a holder. This procedure immobilizes the point of origin of tibialis anticus muscle. The sciatic nerve which was exposed and ligated high in the thigh, was used for direct stimulation through a shielded silver electrode. For direct stimulation of the muscle, the stimuli were applied between the tendon of the muscle and the pin in the femur. The electrical contact with the tendon was made through a wire attached to a saline packed pad of cotton wool. Supramaximal shocks of 0.2 millisecond duration and 0.5 milliseconds duration were employed for indirect and direct stimulation respectively. The interval between the shocks was 10 seconds. The injection of the drugs was made by the distant arterial route (Zaimis, 1951). For distant arterial injection the external iliac artery of the non-operated side was connected with a poly-ethylene cannula pointing towards the Bifurcation of the aorta. The aorta with its cannula was ligated just below the origin of the external iliac arteries. Under such conditions the dose injected was carried directly to the operated leg. The drug solutions used, were made in normal saline. The volume of each injection was 1.0 ml followed immediately by 0.5 ml saline to flush the drugs into the circulation. Actual testing of the drugs was begun after the effect of ether had been dissipated (approx-

mately 2 hours after the operation).

### Drugs

- 1) Acetylcholine chloride (Merck)
- 2) Decamethonium bromide "Syncurine" (Burroughs Wellcome and Co.)
- 3) Succinyl choline chloride "Sucostrin" (Squibb and Company)
- 4) Physostigmine Salicylate (Merck)
- 5) Tetra ethyl pyrophosphate, TEPP (Victor)
- 6) Diisopropyl fluorophosphate DFP (Aldrich Chemical Company)
- 7) d-Tubocurarine chloride (Abbott)

DFP and TEPP were diluted in propylene glycol (Carbowax 200) just before injection.

## ELECTROMYOGRAPHIC PROCEDURES

### Instrumentation

A two channel Electromyograph (TECA Corporation) was used in this work. This instrument has arrangements for voltage calibration (50 uv - 5000 uv/div.), a time base calibration (5 - 1000 milliseconds /div.) and a photographic, tape recording, and loudspeaker display.

Recording of the potentials was made by photographing the oscilloscopic screen with a polaroid camera using a high speed film (speed 3000, type 47). Occasionally the EMC events were taken on the tape recorder for later analysis and photography. In addition, written notes were made during the course of the experiment relative to the general appearance, frequency, amplitude and

the sounds of action potentials.

The pick-up electrodes were made of thin steel needles, 0.2-0.25 mm, insulated up to the very tip which forms the pick-up point. Two needle electrodes were fixed in a small rubber block with a distance between the two pick-up points 5 mm and the depth to which the electrodes can reach after insertion into the muscle, was kept 1 centimeter. Thus in all the experiments this type of bipolar insertion pick-up was used. The ground electrode was attached to the skin of the thigh.

The rabbit was restrained on a wooden board during the routine EMG examination. In a few experiments the animal was suspended in a cloth-sling so that the four limbs could just touch the surface of the table. This arrangement allows the unrestricted movements of the limb during EMG recording.

### Procedure

The bipolar needle electrode was inserted into the muscle under examination. The ground electrode was applied to the skin on the thigh. The pick-up electrode circuit was switched into the electromyograph which had been previously calibrated. Following the insertion of the electrode into the muscle, motor unit potentials usually appear. However, when the animal is quieted the potentials usually disappear. Beginning with "quiet" or zero activity action potentials were recorded during various conditions of muscle functions.

Electromyographic responses of the muscle to drugs in

advanced local tetanus and chronic denervation were recorded following their administration by distant arterial route as described earlier.

Action potentials from the sciatic nerve were also obtained in a few experiments. The spinal cord of the rabbit was sectioned between T<sub>6</sub>-T<sub>12</sub> under preliminary ether. Next, the sciatic nerve was exposed by a longitudinal incision in the thigh. The nerve was carefully cleared from the surrounding connective tissue. The pick-up electrode was inserted into the nerve trunk and held in position by means of an electrode holder. The exposed nerve was kept in a pool of mineral oil formed by filling the incision in the thigh with the oil. The temperature of the oil was maintained at body temperature by a heating lamp. Any activity in the nerve was recorded while it was at rest and during stimulation.

### ELECTRODIAGNOSIS (EDX)

#### Instrumentation

The instrument used in this study was a constant current chronaxie meter (MEDITRON) or more specifically, a square wave constant current impulse generator. The duration of the impulses could be varied from 0.1 milliseconds to 1000 milliseconds and the interval between the pulses from 1 second to 4 seconds. The current out-put of the generator was measured by a highly sensitive well damped DC milliammeter. The stimulating electrodes consisted

of one quarter inch copper rod insulated to within one-half inch of the tip. The bare tip was covered by a piece of chamois. This chamois tip moistened with saline formed the stimulating electrode. The indifferent electrode consisted of another strip of chamois to which the wire connections were made.

The rabbit was restrained in a specially prepared wooden board. The limb under examination was immobilized upon a small platform by means of elastic bands. This arrangement keeps the animal not only quiet but comfortable too, during the entire period of examination. (Fig. I)

#### Design of the experiment

Preliminary EMG studies on local tetanus indicated that fibrillation-like potentials make their appearance around 8-12 days following the injection of the tetanus toxin. It is well documented that fibrillation potentials also appear in chronically denervated muscle around 4-7 days following the sectioning of the motor nerve. Since in the pilot experiments the EMG pattern of the local tetanus was almost identical to that of chronic denervation it was thought desirable to conduct a systematic electrodiagnostic study on one group of animals with local tetanus and another group of denervated animals simultaneously.

A group of eight rabbits for local tetanus and another group of six rabbits for chronic denervation formed the subject of this study. The untreated and non-operated contralateral sides were taken as control for each animal. All the animals in each

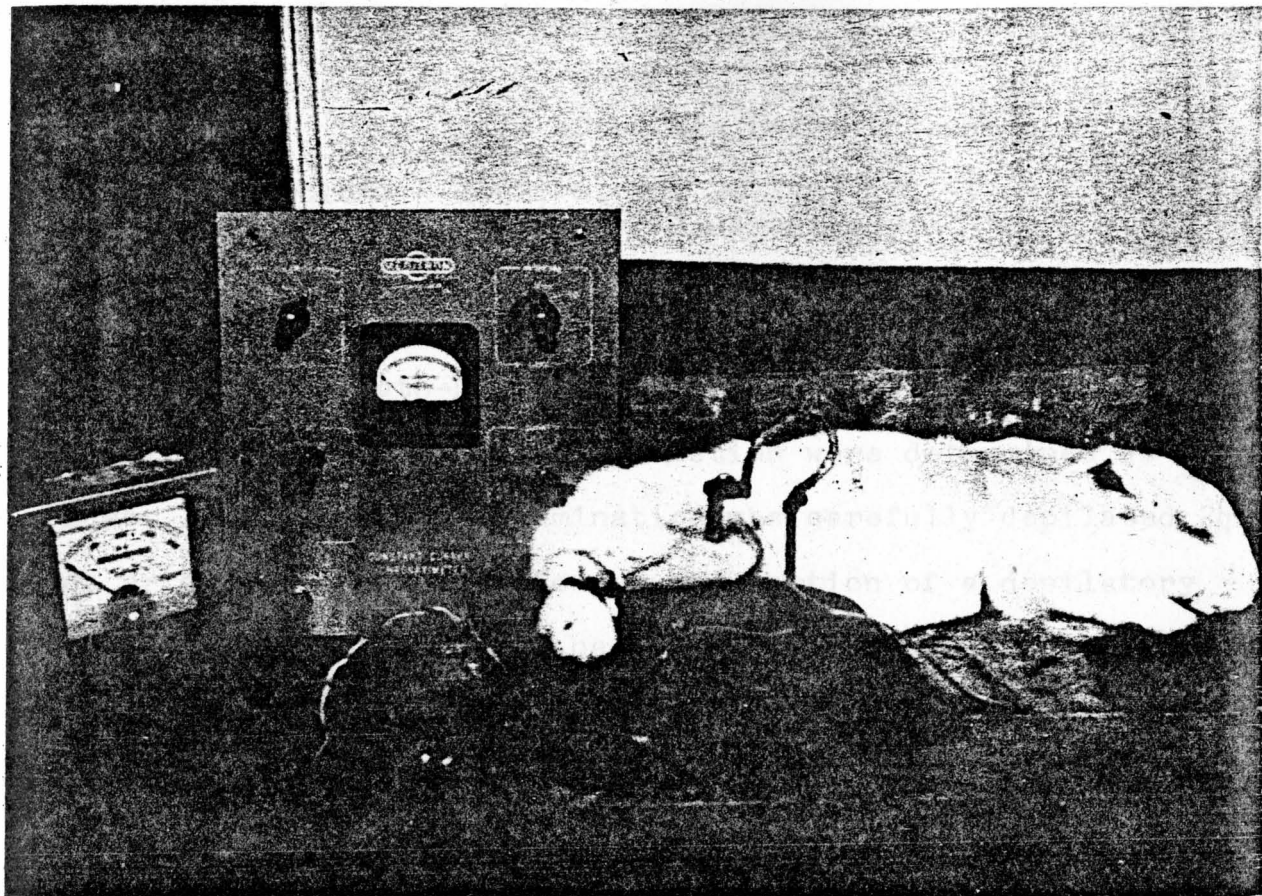


Figure 1

Setup for electrodiagnostic examination

group were examined on the same day. Each group was examined during the course of eight weeks. The animals were examined according to the following schedule, based on pilot experiments.

Local tetanus: 1, 3, 5, 8, 12, 20, 30, 45, 55  
days after the toxin injection

Chronic denervation: 1, 2, 6, 12, 20, 30, 45, 55  
days after denervation

### Strength-Duration curves

The Strength-Duration curves of normal muscle and those in local tetanus and chronic denervation were determined as follows. The limb under examination was carefully depilated 24 hours prior to the examination by application of a depilatory mixture (mixture of soap and barium sulphide) and washed carefully. The animal was restrained on the board. The positive terminal of the stimulator was connected to the indifferent electrode and the negative electrode was positioned over the motor point (per cutaneous stimulation). The stimulator was set to deliver repetitive cathodal stimuli with a duration of 300 millisecond at an interval of 1000 milliseconds. Next, the current control was advanced until a perceptible muscle contraction can be seen. The position of the electrode was moved around the motor point region until the lowest threshold reading was obtained. This point of stimulation was used throughout the Strength-Duration curve study. The complete Strength-Duration curve was then, run with successive decrements in the stimulus duration in the following order: 100, 60, 30, 10, 6, 3, 1, 0.6, 0.3, 0.1 milliseconds. Plots were made on



semi-logarithmic paper.

### Galvanic-tetanus ratio

With the stimulus interval dial at 2000 milliseconds and the duration dial at 1000 milliseconds the threshold current necessary for a quick twitch, as the current is made, and that for a persistent contraction during the whole stimulus duration, when the current is increased, was obtained. The quotient obtained by dividing the tetanus current by twitch current gives the galvanic-tetanus ratio.

### Response to repetitive stimuli

The current required to produce a sustained contraction, of tetanic type under the following three different frequencies of stimulation was determined.

- 1. millisecond duration, 1. millisecond interval(500 CPS)
- 1. millisecond duration, 5. millisecond interval(166 CPS)
- 1. millisecond duration, 10. millisecond interval(91 CPS)

## CHAPTER III

### EXPERIMENTAL RESULTS

#### Local Tetanus

Typical symptoms of local tetanus appeared 36-48 hours after the injection of tetanus toxin into the muscle or the nerve supplying the muscle. In the present work local tetanus was produced in the tibialis anticus muscle by injecting 200 guinea pig MLD/kg of tetanus toxin. The first sign of local tetanus is slight stiffness of the affected limb. By the third day the stiffness became progressive and the leg was quite stiff at the ankle joint and held extended almost at  $120^{\circ}$  angle. The affected limb exhibited a striking picture suggestive of reflex hyperactivity. Any kind of weak stimulus to the affected limb produced a vigorous increase in the extension of the limb. This state of hyper-reflexia lasted for about 4-5 days and gradually disappeared. The animal no longer showed the signs of hyper-reflexia even with a stronger stimulus like pinching and pricking of the affected limb. However, the lasting stiffness or the rigidity of the limb continued to remain for almost 6-8 weeks after which time the rigidity disappeared and the animal regained its ability to use the muscle.

These signs of local tetanus were always confined to the affected limb or organ and the animal looked essentially normal except for the affected limb. (Fig. 2)

In a few pilot experiments it was observed that with a

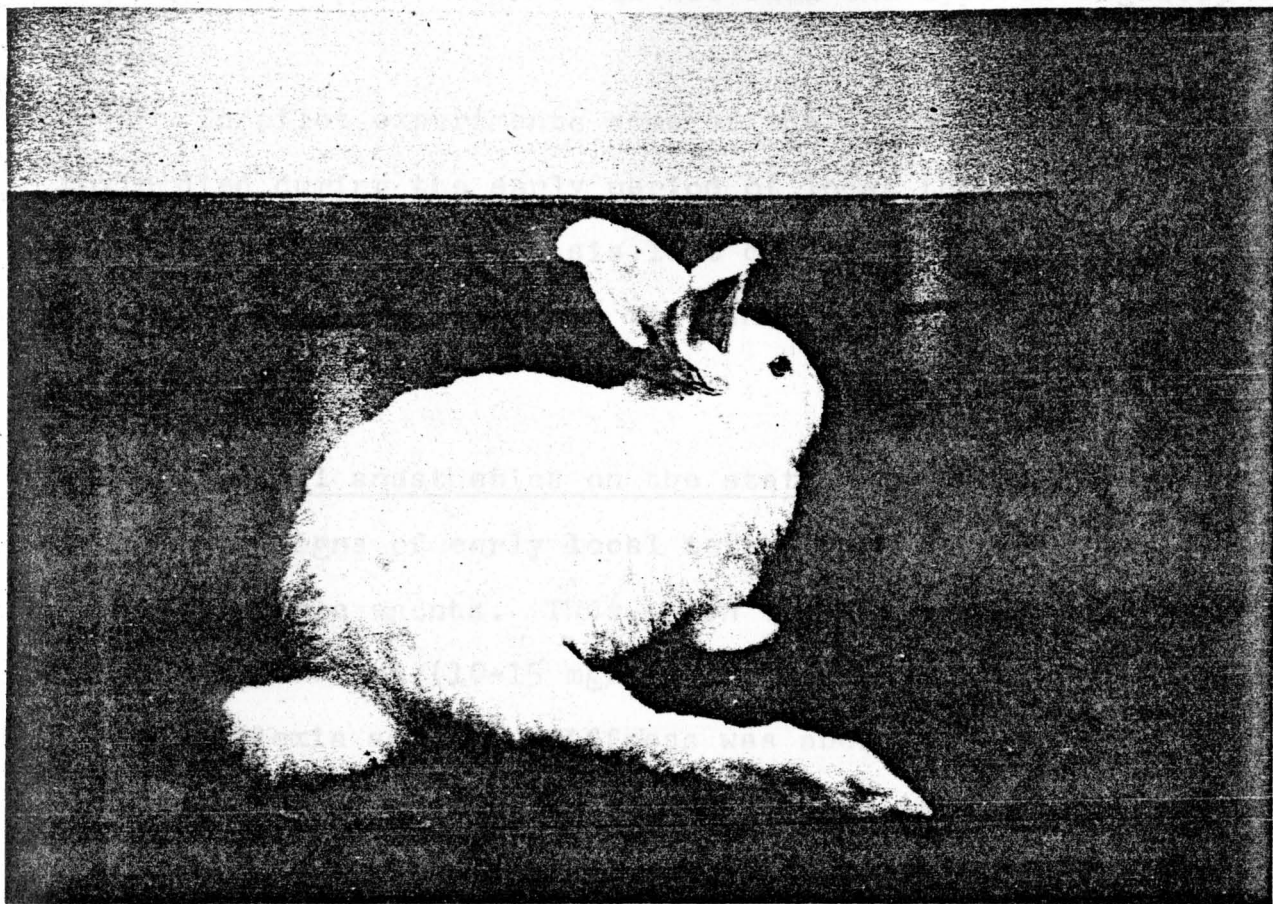


Figure 2

Rabbit in local tetanus

higher dose of toxin, the hyper-reflexia appeared to spread to the opposite limb. Probably this was reflex action, for on longer observation the opposite limb did not show the typical rigidity of the limb.

In pilot experiments some of the animals with high dose of toxin died during the early period of local tetanus. Those which survived this early crisis lost considerable weight. This was found to be due to the fact that these animals could not take their food.

#### Effect of general anesthetics on the status of local tetanus

The signs of early local tetanus can be abolished by general anesthetic agents. Thus, when a sub-threshold anesthetic dose of pentobarbital (10-15 mg/kg) was administered intravenously the hyper-reflexia and the stiffness was abolished and returned after the effect of the anesthetic was over. As mentioned before, this abolition of stiffness and in a few animals the softening of the rigidity, was found only during the first 4 to 5 days after the toxin injection. Once this stage is past, the rigidity (long term rigidity) of the limb could not be abolished or suppressed by any dose of pentobarbital.

#### Effect of nerve sectioning on local tetanus

Intact motor nerve connection plays a very important part in the manifestations of the symptoms of local tetanus. In 6 rabbits the right sciatic nerve was sectioned under ether and 200

guinea pig MLD/kg of toxin was injected immediately into the tibialis muscle. In none of these animals were the symptoms of local tetanus seen even one week after the injection. However, six control rabbits showed local tetanus within 36-48 hours as described before.

#### Sectioning of motor nerve after the onset of local tetanus

In order to determine whether or not motor nerve plays a role in the existing local tetanus, the following sets of experiments were performed at different time intervals after the injection of tetanus toxin. In a group of nine animals local tetanus was produced as before, by intramuscular injection of tetanus toxin. These animals were divided into three subgroups. In the first subgroup the sciatic nerve was sectioned two days after the injection of toxin, i.e. when clear symptoms of local tetanus were present. Sectioning of the motor nerve completely abolished the hyperreflexia and the apparent rigidity. These rabbits could not be distinguished from denervated rabbits without previous local tetanus, i.e. no subsequent rigidity appeared.

In the second subgroup of 3 rabbits sciatic nerve was sectioned 4 days after the injection of toxin, i.e. at least 2 days after the onset of typical signs of local tetanus. Here again, the appearance of the limb after nerve sectioning was essentially the same as that noted in the first subgroup.

In the third subgroup, however, the nerve sectioning was done 8 days after the injection of toxin, i.e. at least 6 days

after the onset of local tetanus. In this group, the appearance of the limb after nerve sectioning was strikingly different from the previous two subgroups. Thus, the rigidity of the limb, which was present all along did not disappear, nor did it lessen. These observations, therefore, clearly indicated that the local tetanus passes through at least 2 different phases during this course of time. The first stage (4-5 days) was marked by the hyperreflexive nature of the affected limb. This hyperreflexia and the accompanying stiffness can be completely abolished or suppressed to a large extent by anesthetics and nerve sectioning. The second stage was marked by the presence of unremitting rigidity even after the administration of anesthetics and nerve sectioning.

#### Electromyographic findings in local tetanus and their comparison with chronic denervation

Preliminary electromyographic findings with advanced local tetanus indicated a close similarity to the findings in chronic denervation. Furthermore, since such observations had not been reported by previous investigators, a systematic EMG study was considered to be necessary. An EMG comparison between normal muscles and muscles in various phases of local tetanus and chronic denervation should reveal certain information on the pathogenesis of local tetanus. Such information hitherto was not reported in the literature.

Electromyographic features in normal rabbits

The characteristic EMG features of normal rabbits were found to be the following: with the needle electrode in the muscle belly (tibialis anticus) and the rabbit at rest, the characteristic EMG feature was "electrical silence", i.e. the base line on oscilloscope remained straight and no electrical potentials or motor unit potentials were produced.

When the muscle was made to contract minimally, simple electrical potentials called "motor units" were obtained. In this type of recording system biphasic motor unit potentials were the usual and consistent findings. The electrical parameters of the motor unit potentials were: amplitude 200-1200 uv, duration 3-5 milliseconds, phases 2 (biphasic), occasionally 3-4 phases were obtained. The sound of the potentials was dull and characteristic. These motor units increased in number (discharge frequency), amplitude, and also additional motor units were brought into action when the limb was made to contract increasingly by mechanical or reflex action. This increased electrical activity produced by a moderate contraction, resulted in a pattern consisting of many motor unit potentials. This is known as "partial interference pattern." On the other hand, with a strong contraction, a summation or "interference pattern" was obtained. In the interference pattern, the base line was completely obliterated. However, there was a return to the normal base line as soon as the muscle was allowed to relax.

### Electromyographic features in local tetanus

Electromyographic records from muscle in local tetanus exhibited several distinctive features. The clinical evidence of hyperactivity and hyperreflexia of the muscle was closely paralleled by the changes in the electromyograms obtained from the affected muscle. One significant feature was the exaggeration and prolongation of electrical activity after any weak stimulus like touch or sound. Though the reaction in a normal muscle subsides almost immediately after the termination of the stimulus, the electrical activity in the muscle in local tetanus persisted with a slow decrement over several seconds and some times even for minutes. During the peak of the hyperreflexia phase (3-5 days) the EMG responses were very easily evoked, until eventually, they became continuous and apparently spontaneous. Electrical silence was rarely achieved. The parameters of the motor unit potentials when discharged at moderately low frequency, were found to be essentially the same as those from normal muscle during moderate degree of contraction. The parameters of the electrical potentials were - amplitude 400-1600 uv, duration 3-6 milliseconds, phases 2-4. The sound was characteristic and rattling. (Fig. 3 a,b)

An interesting electromyographic observation followed immediately after this hyperreflexia phase. As described earlier, the muscle gradually passed into a stage of so-called "myostatic contracture". At this transitional stage the exaggerated hyper-reflexive nature was considerably reduced, even though, the



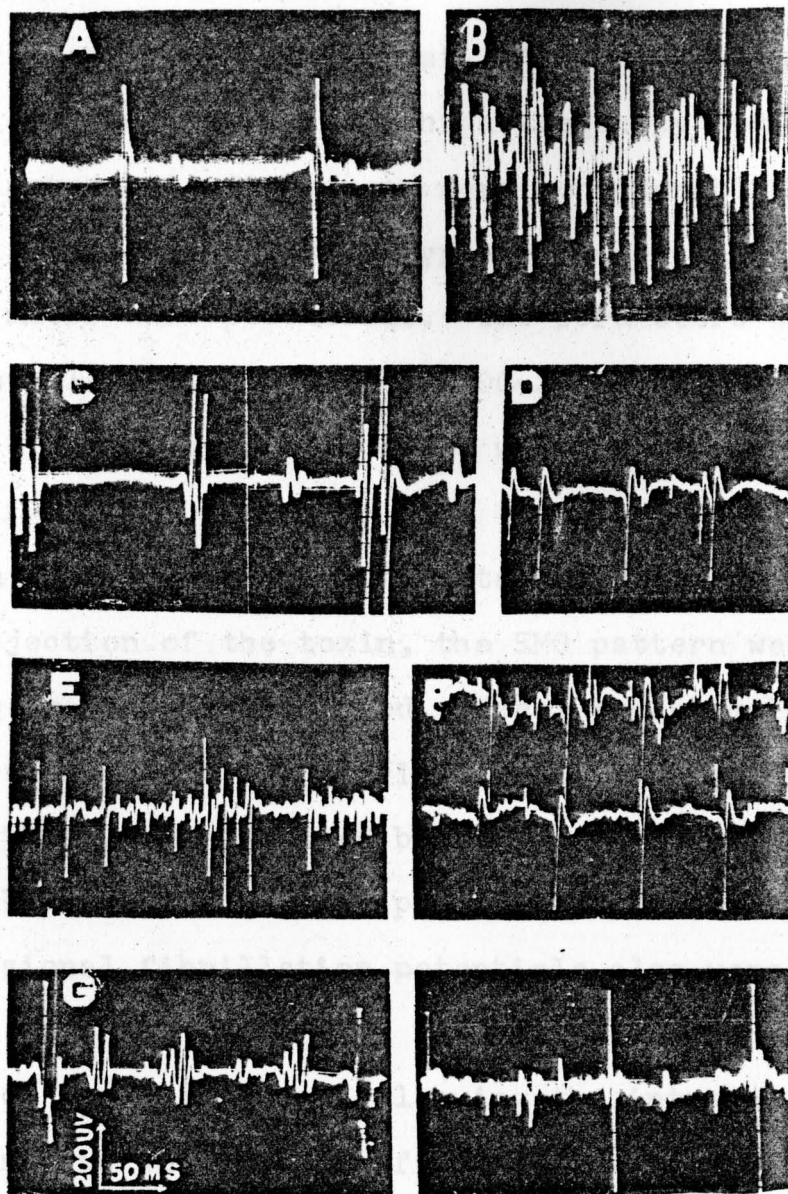


Figure 3

Electromyographic pattern during various stages of local tetanus

- A. Normal motor unit potentials B. Interference pattern in early local tetanus C. Polyphasic potentials D. Positive sharp waves E. Fibrillation potentials F. Positive sharp waves G. Polyphasic potentials. H. Normal motor unit potentials

rigidity still continued to persist. A concomitant change in the EMG pattern occurred in which the high frequency and apparently spontaneous motor unit discharges were considerably reduced and in their place a large number of polyphasic potentials appeared along with normal motor unit potentials. The parameters of the polyphasic potentials were - amplitude 400-1200 uv, duration 5-15 milliseconds, phases 4-6. The sound was rough and rasping. (Fig. 3 c)

In the early phase of myostatic contracture, 6-9 days after the injection of the toxin, the EMG pattern was different from the one previously described. Here, the normal motor unit potentials and also the polyphasic potentials seemed to lose their integrity. These were replaced by a number of ill defined potentials, resembling the so-called positive sharp or V-wave potentials. Occasional fibrillation potentials also were present. (Fig. 3 d)

From about ten days following the intra-muscular injection of tetanus toxin and thereafter, the major EMG finding consisted of complete fibrillation potentials. These were spontaneous and could be picked up from the entire length of the affected muscle. These fibrillation potentials were characterized by the following parameters - voltage 50-400 uv, duration 0.5-2 milliseconds. They were biphasic sharp spikes. The sound was like high pitched sharp clicks. Immediately following the insertion of electrodes in the muscle there was a burst of fibrillation poten-

tials which gradually came to a steady rate of discharge. Sometimes the discharge was rhythmic, i.e. waxing and waning in the frequency of their discharge. Passive or mechanical movements of the limb did not produce any significant change in the nature or frequency of these fibrillation potentials. The animal did not possess any control over this spontaneous discharge. (Fig. 3 e)

This phase of spontaneous fibrillation potentials continued to last for almost 4-5 weeks after the onset of first signs of local tetanus. At the end of this period the EMG features were essentially a reverse sequence of events, with passing of time, i.e. the fibrillation potentials were replaced by V-waves and accompanying polyphasic potentials. Normal motor units were the last to appear. This last phase of local tetanus was a phase of recovery and corresponded closely to clinical picture of recovery. Eventually the rabbits regained their ability to use their muscles. (Fig. 3 f,g,h)

In a total of 18 rabbits subjected to long term examination, 4 rabbits showed a 2-3 weeks delay in their recovery judged on the basis of the clinical appearance of the muscle. However, the electromyogram was entirely normal during this period.

Further evidence with respect to the true nature of the fibrillation potentials was obtained from the following experiments. It is well documented that fibrillation potentials are typical of chronic denervation. They are independent of central nervous system influence, originate in the muscle fibre itself and

are unaffected by central depressants and anesthetic agents.

In a group of 3 rabbits with local tetanus established for 15 days and which showed spontaneous fibrillations on electromyograph, intravenous injection of 35 mg/kg of pentobarbital was given. The electromyogram before, during and after the administration of pentobarbital remained essentially unchanged. (Fig. 4)

In another group of 3 rabbits the sciatic nerve supplying the muscle in local tetanus (15-20 days) was exposed under ether anesthesia, and a lifting ligature was placed around the sciatic nerve. While a continuous EMG recording was being made, the sciatic nerve was sectioned. No change in the electromyogram was seen. These findings therefore, indicated that these fibrillation potentials of advanced local tetanus were independent of central nerve supply and had their origin in the muscle fibre itself.

#### Electromyographic features in chronic denervation

In severe peripheral nerve lesion in human or in experimental animals, undergoing a Wallerian degeneration, the EMG findings are well documented. The time of onset of fibrillatory activity vary, depending upon the animal species and the distance of the nerve lesion from the muscle under investigation. Thus, for instance, fibrillation potentials appear after 2-3 days in rats, 5-7 days in rabbits and cats, 7-8 days in dogs after the nerve sectioning. In the case of frogs, several weeks may elapse before any consistent and persistent fibrillatory activity is seen.

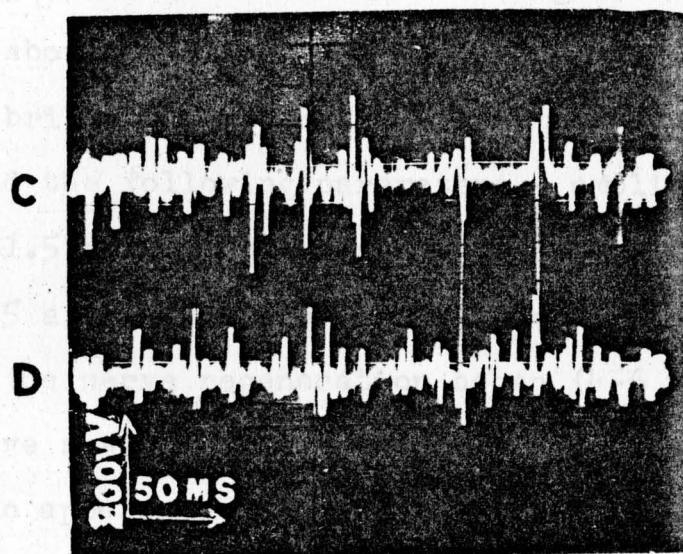
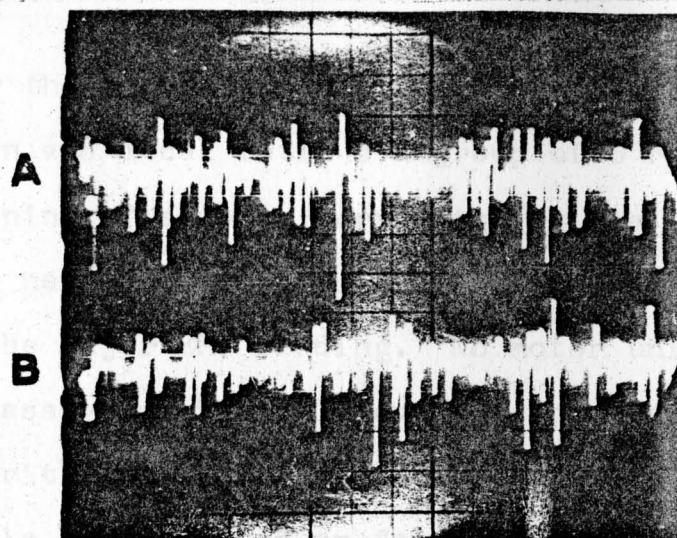


Figure 4

Effect of pentobarbital on the spontaneous fibrillation potentials  
in local tetanus and chronic denervation

Upper - Local Tetanus

A - before pentobarbital

B - after pentobarbital

Lower - Chronic Denervation

A - before pentobarbital

B - after pentobarbital

For the purposes of comparison of electrical potentials in denervation and local tetanus a group of 6 rabbits were denervated by sectioning of sciatic nerve high in the thigh. Immediately following the nerve section and 2-3 days thereafter, electrical silence was the major EMG finding. No motor unit potentials were produced by passive mechanical movements of the limb. However, transient "irritation potentials" or so-called "insertion activity" were obtainable on the third or fourth day. On the fourth or fifth day spontaneous fibrillation potentials made their appearance, at first - a few and then increasing in number. From the sixth day to about four weeks, a large number of spontaneous and persistent fibrillation potentials were the major finding. These potentials had the following parameters - amplitude 50-350 uv, duration 0.5-1.5 milliseconds, phases usually 2, (biphasic sharp spikes, Fig. 5 a). Sound was that of sharp high pitched click.

As the nerve regeneration began (4-6 post-operative weeks) positive sharp V-waves, and occasional polyphasic potentials began to appear on mechanical stimulation of the limb or purposeful contraction on the part of the animal. This was followed by the appearance of normal motor units. In all the rabbits in this group, regular biphasic motor units potentials were not observed until about 7-8 weeks following the nerve section. The reappearance of normal motor units potentials represents electromyographically, a sign of normal reinnervation.

(Fig. 5 b,c,d)



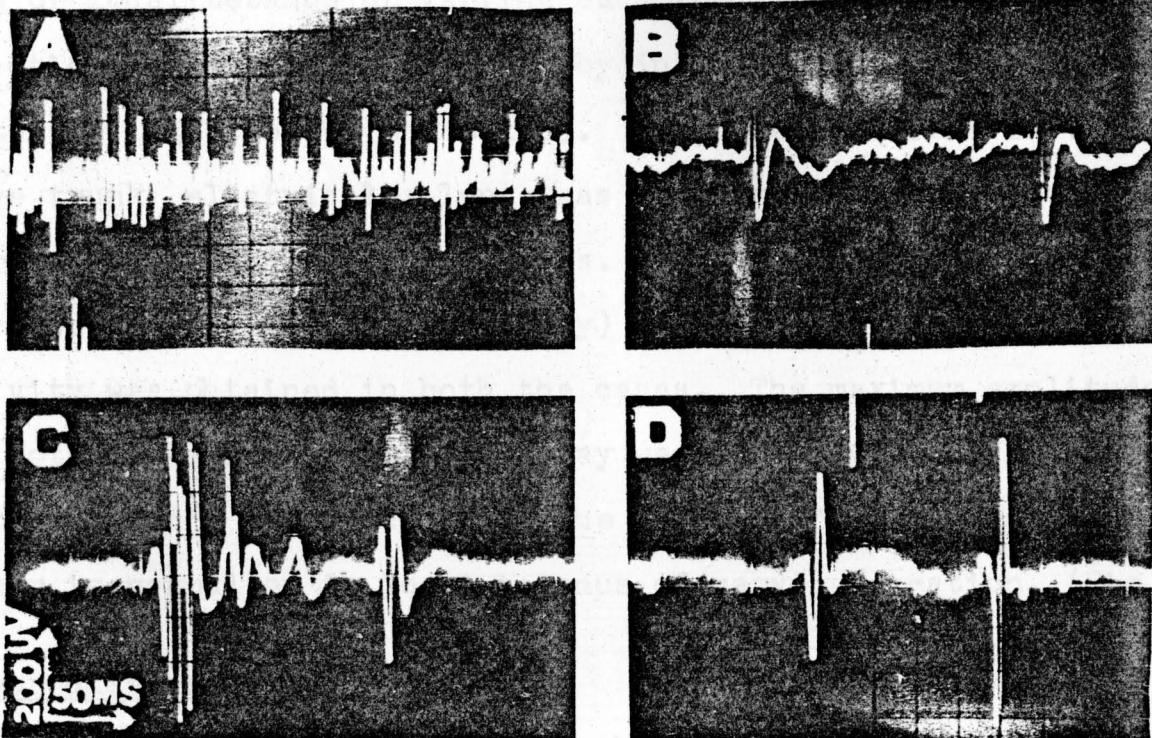


Figure 5

Electromyographic pattern in chronic denervation

- |  |   |
|--|---|
| A - Fibrillation potentials<br>(20 days) | B - Positive sharp waves<br>(20 days)         |
| C - Polyphasic potentials<br>(40 days)   | D - Normal motor unit potentials<br>(40 Days) |

### Electrical activity of the nerve supplying the muscle in local tetanus

Electrical potentials recorded from the sciatic nerve trunk of normal rabbits were compared with those obtained in the case of local tetanus of varying duration, 2-15 days following the injection of tetanus toxin (both by intramuscular and intraneural routes of injection of the toxin). With the electrode in the nerve trunk electrical silence was the normal finding both in normal as well as in local tetanus. On the other hand when the nerve was stimulated (mechanically) a high degree of electrical activity was obtained in both the cases. The maximum amplitude of the potentials recorded in this way was about 30 uv. No significant difference was observed in the pattern of electrical activity either in normal or in local tetanus of varying duration. (Fig. 6)

### Electrodiagnosis

Results of electromyographic studies from the muscle in local tetanus and chronic denervation indicated a parallelism between these two phenomena. It was therefore, thought desirable to conduct an additional study of certain electrophysiological properties of peripheral nerves and muscles. Such electrophysiological examination presents both qualitative and quantitative information on the nature of the neuromuscular apparatus. In this section results of an electrodiagnostic study comprising - rheobase, chronaxie, strength-duration curves, galvanic-tetanus ratio and responses to repetitive stimulation of muscle in local



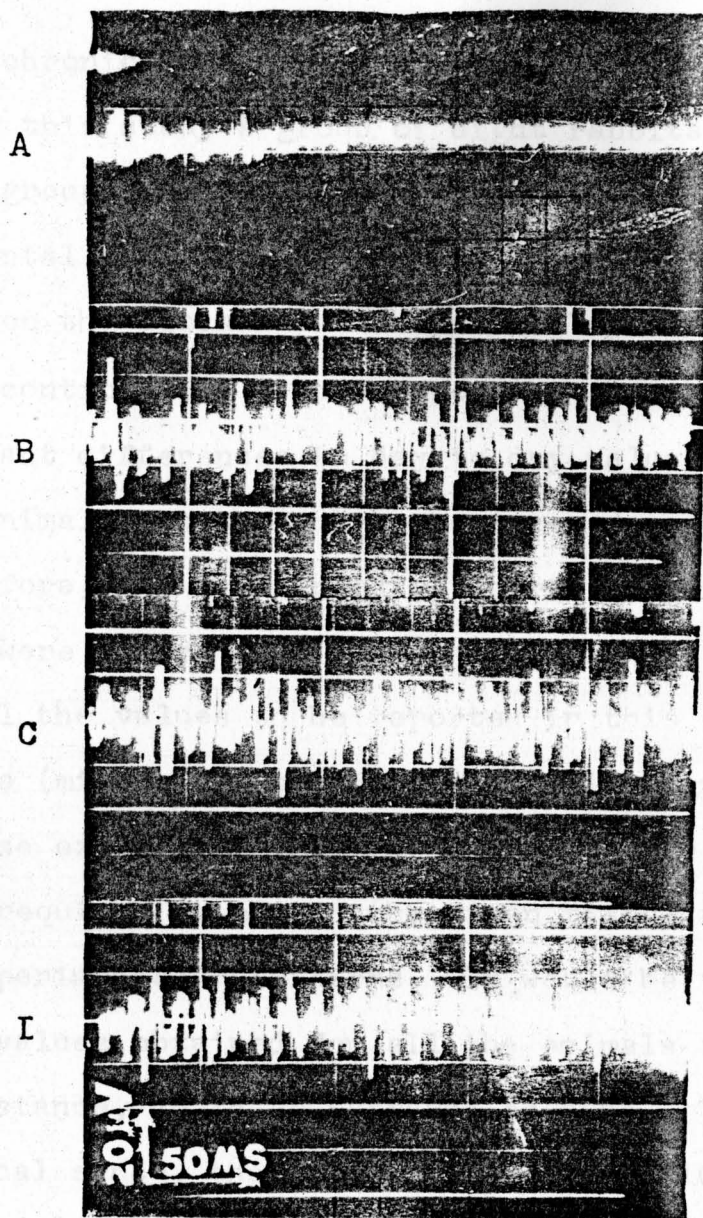


Figure 6

Electrical activity recorded from the nerve trunk of rabbit in local tetanus

- A - In normal animal at rest
- B - In normal animal during stimulation
- C - In local tetanus (3 days) during stimulation
- D - In local tetanus (15 days) during stimulation

tetanus and chronic denervation is presented.

In this study a group of eight rabbits for local tetanus and another group of six rabbits for chronic denervation formed the experimental subjects. Since preliminary electrodiagnostic examination on the tibialis anterior muscle of normal animals and that of the control side of the experimental subjects did not show any significant differences in day to day values, a separate group of control animals was thought to be unnecessary. However, as mentioned before, the corresponding untreated limbs of experimental subjects were always used for control.

All the values to be reported in this section are in units of time (milliseconds and current (milliamperes). In essence, these experiments consist in noting the amount of electrical current required to excite the given tissue under a set of specified experimental conditions. It would be very convenient if the current values obtained for all the animals for a given duration, for instance, could be averaged as such. This however, is not too logical since, the current threshold values vary from animal to animal, at least, by a small factor. However, the repeated determinations from each animal gave almost the same values. In order to make a logical comparison among each group of animals and a statistical evaluation, Threshold Ratio (T.R.) was employed. This may be explained as follows:

$$\text{Threshold Ratio} = \frac{\text{Threshold current (milliamperes) at a specified duration}}{\text{threshold current (milliamperes) at infinite duration}}$$

For practical purposes, a duration of 300 milliseconds is considered by most electrophysiologists, to be "infinite" duration, and the threshold value at this duration of the stimulus is known as rheobase. All the numerical values were, therefore, first converted into threshold ratios wherever necessary and then averaged.

### Rheobase

Rheobase values of the tibialis anticus muscle of the control, local tetanus and denervated animals were averaged separately for each of the groups on each day of examination. They are tabulated in Table I. These values are plotted in Fig. 7.

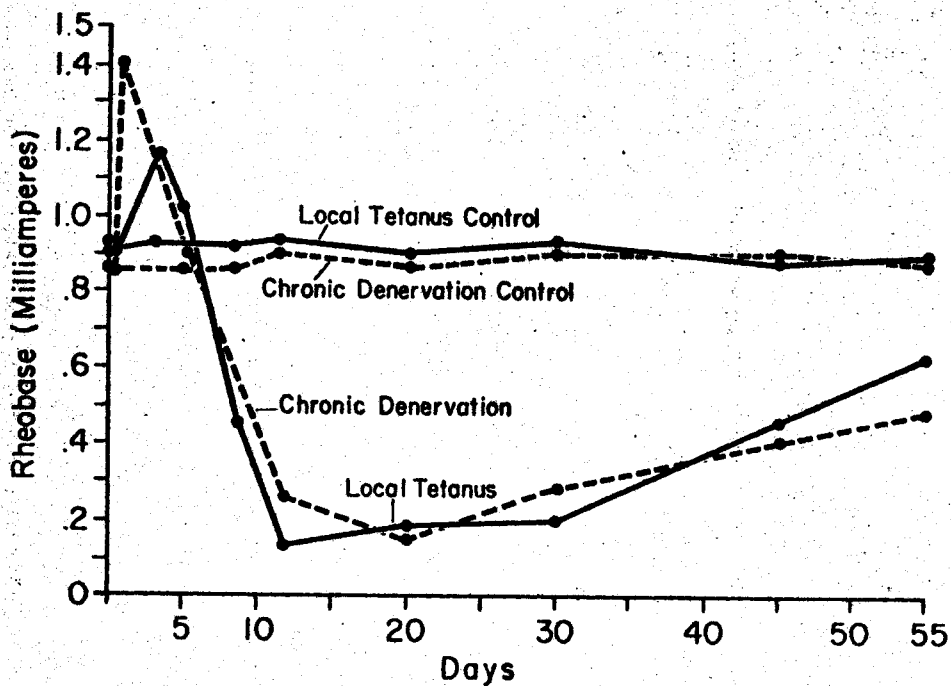
### Strength-Duration curves

The strength-duration curves were determined on muscle from a group of eight rabbits in local tetanus and another group of six rabbits in denervation. The examinations were made according to the schedule given in the Materials and Methods section. All the animals in each group were examined on the same day so that the data were homogeneous (all local tetanus animals on one day and all denervated ones on the following day). Actual numerical values were converted into threshold ratios as previously discussed. The threshold ratios for each duration were averaged and their standard deviation was computed. This gave one single curve for local tetanus and a second curve for denervation. The averaged threshold ratio values along with their standard deviations are tabulated in Tables II and III and the plots of these values are

TABLE I

## RHEOBASE IN LOCAL TETANUS AND CHRONIC DENERVATION

Days after toxin injection or nerve section	Local Tetanus (8 animals)		Chronic Denervation (6 animals)	
	Avg.	S.D.	Avg.	S.D.
1	0.88	± .17	.85	± .22
2			1.45	± .14
5	1.06	± .15		
6			.91	± .11
8	0.45	± .21		
12	0.13	± .03	.28	± .12
20	0.19	± .22	.15	± 0.09
30	.20	± .07	.28	± .11
45	0.47	± .18	0.42	± .10
55	0.62	± .21	.48	± .23
Control	0.92	± .18	0.86	± .20



### Rheobase in Local Tetanus and Chronic Denervation

Each point on the curve represents the mean from observations on 8 animals (Local Tetanus) and on 6 animals (Chronic Denervation).

Figure 7

## STRENGTH-DURATION DATA IN LOCAL TETANUS (8 ANIMALS)

Duration ms	Control Side		Days After the Injection of Toxin									
			1 Day		3 Days		5 Days		8 Days			
	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>
300	1.00		1.00		1.00		1.00		1.00		1.00	
100	1.00		1.00		1.00		1.00		1.00		1.00	
60	1.00		1.00		1.00		1.00		1.00		1.00	
30	1.00		1.00		1.00		1.00		1.00		1.00	
10	1.00		1.00		1.04+	.24	1.00		1.90+	.13		
6	1.04+	.07	1.02		1.13+	.38	1.25+	.21	2.61+	.21		
3	1.11+	.89	1.12+	.29	1.41+	.64	1.60+	.84	5.66+	.24		
-1	1.36+	.22	1.34+	.18	2.03+	.86	2.67+	.62	9.00+	1.13		
0.6	1.58+	.26	1.61+	.15	2.59+	.32	3.49+	.40	10.76+	1.54		
0.3	2.18+	.42	2.15+	.15	3.43+	1.84	4.52+	.74	13.70+	2.01		
0.1	3.55+	.83	3.54+	.23	5.69+	2.87	6.38+	.88	21.12+	2.93		
		<u>12 Days</u>		<u>20 Days</u>		<u>30 Days</u>		<u>45 Days</u>		<u>55 Days</u>		
	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>	<u>T.R.</u> <u>Avg.</u>	<u>S.D.</u>
	1.00		1.00		1.00		1.00		1.00		1.00	
	1.00		1.00		1.00		1.00		1.00		1.00	
	1.00		1.35+	.13	1.44+	.30	1.00		1.00		1.00	
	1.52+	.64	1.97+	.18	2.37+	.97	1.44+	.63	1.00		1.00	
	5.27+	.79	4.90+	.43	4.66+	2.08	2.88+	.34	1.47+	.33		
	7.70+	1.36	7.39+	.66	7.29+	2.79	4.07+	.68	2.43+	.48		
	13.77+	2.07	13.70+	1.84	11.30+	3.57	5.72+	.32	2.86+	.42		
	30.63+	3.34	23.45+	4.02	17.60	8.94	7.92+	.53	3.15+	.58		
	43.42+	3.25	37.30+	7.51	23.96	4.01	9.38+	7.25	4.19+	.68		
	73.23+	6.48	48.02+	12.36	38.03	4.66	11.56+	1.45	5.77+	.78		
	145.49+	27.69	80.31+	17.52	67.28	13.41	18.36+	2.45	8.71+	1.23		

T.R. = Threshold Ratio

S.D. = Standard Deviation

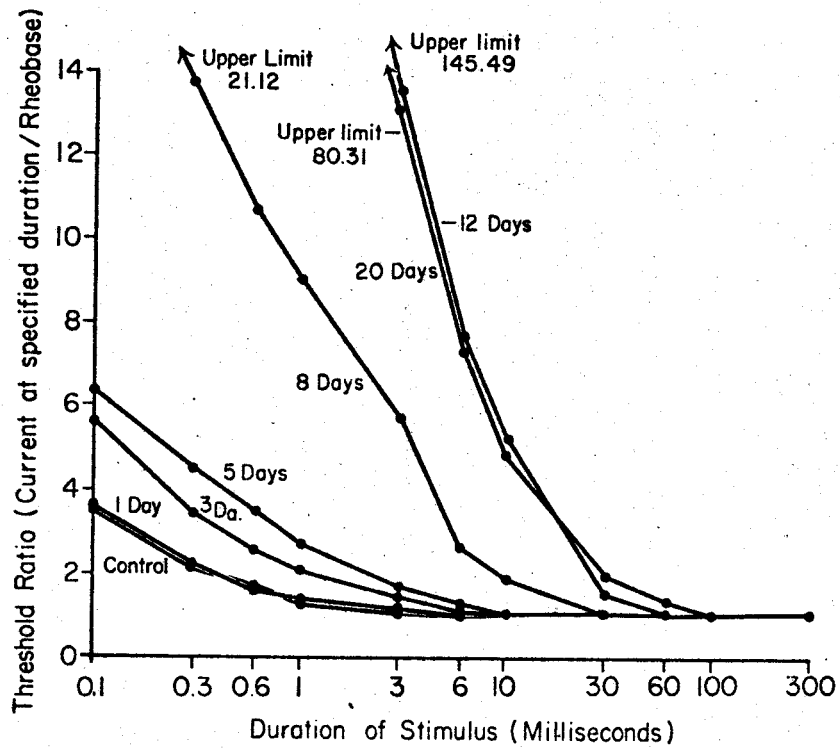
TABLE III

## STRENGTH-DURATION DATA IN CHRONIC DENERVATION (6 ANIMALS)

Duration ms	Control Side		DAYS AFTER DENERVATION							
			1 Day		2 Days		6 Days		12 Days	
	<u>T.R.</u>	<u>S.D.</u>	<u>T.R.</u>	<u>S.D.</u>	<u>T.R.</u>	<u>S.D.</u>	<u>T.R.</u>	<u>S.D.</u>	<u>T.R.</u>	<u>S.D.</u>
	<u>Avg.</u>		<u>Avg.</u>		<u>Avg.</u>		<u>Avg.</u>		<u>Avg.</u>	
300	1.00		1.00		1.00		1.00		1.00	
100	1.00		1.00		1.00		1.00		1.00	
60	1.00		1.00		1.00		1.00		1.00	
30	1.00		1.00		1.00		1.00		1.53+	.34
10	1.00		1.07+	.08	1.13+	.17	1.17+	.15	3.32+	.78
6	1.17+	.26	1.29+	.34	1.30+	.27	1.55+	.16	4.94+	.17
3	1.23+	.25	1.41+	.36	1.59+	.39	2.11+	.26	8.63+	1.36
1	1.42+	.23	1.51+	.34	2.56+	.44	3.70+	.27	14.17+	3.49
0.6	1.66+	.25	1.79+	.33	3.32+	.51	5.28+	.17	21.98+	3.75
0.2	2.16+	.33	2.16+	.33	4.82+	.47	7.56+	2.06	37.23+	6.19
0.1	3.34+	.53	3.61+	.51	10.61+	.47	17.57+	4.64	73.55+	8.18
	20 Days		30 Days		45 Days		55 Days			
	<u>T.R.</u>	<u>S.D.</u>	<u>T.R.</u>	<u>S.D.</u>	<u>T.R.</u>	<u>S.D.</u>	<u>T.R.</u>	<u>S.D.</u>	<u>T.R.</u>	<u>S.D.</u>
	<u>Avg.</u>		<u>Avg.</u>		<u>Avg.</u>		<u>Avg.</u>			
	1.00		1.00		1.00		1.00			
	1.00		1.00		1.00		1.00			
	1.37+	.16	1.26+	.11	1.00		1.00			
	1.84+	.44	1.65+	.16	1.17+	.15	1.00			
	4.62+	.68	3.49+	.33	1.88+	.17	1.32+	.18		
	6.85+	.99	4.84+	.07	1.91+	.42	2.21+	.23		
	10.60+	.82	7.58+	.55	4.74+	.44	4.60+	.25		
	21.95+	1.08	16.00+	3.17	7.27+	1.82	8.15+	1.34		
	30.18+	2.40	23.00+	1.72	12.46+	2.82	12.23+	1.85		
	53.90+	2.90	36.92+	5.77	20.76+	.32	16.52+	2.31		
	114.20+	2.29	77.10+	8.06	35.35+	.58	25.00+	2.85		

T.R. = Threshold Ratio

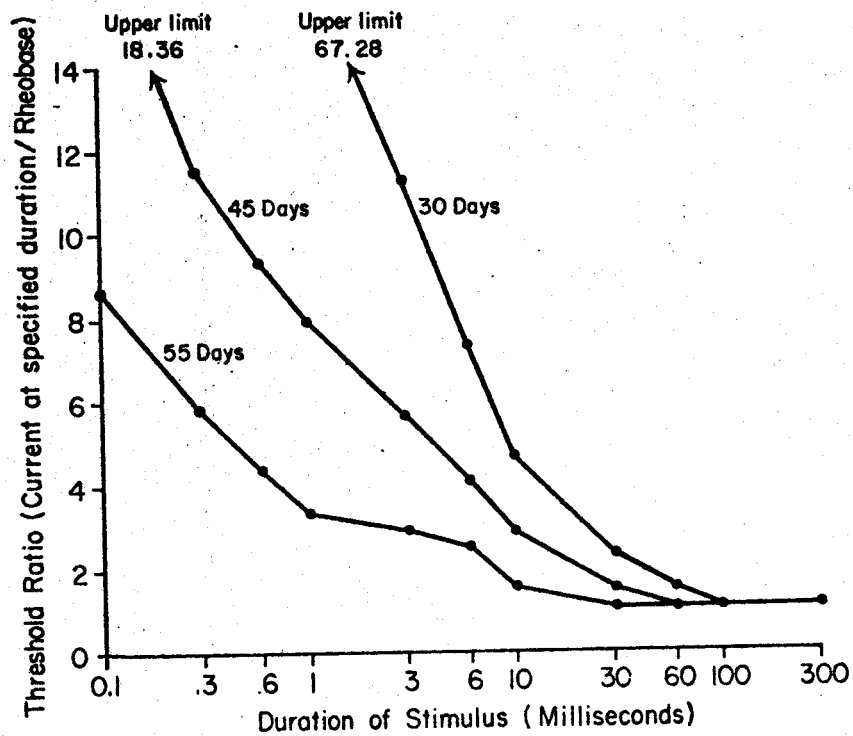
S.D. = Standard Deviation



**Strength Duration Curves in Local Tetanus**  
 Each point represents the mean from the observations on 8 animals

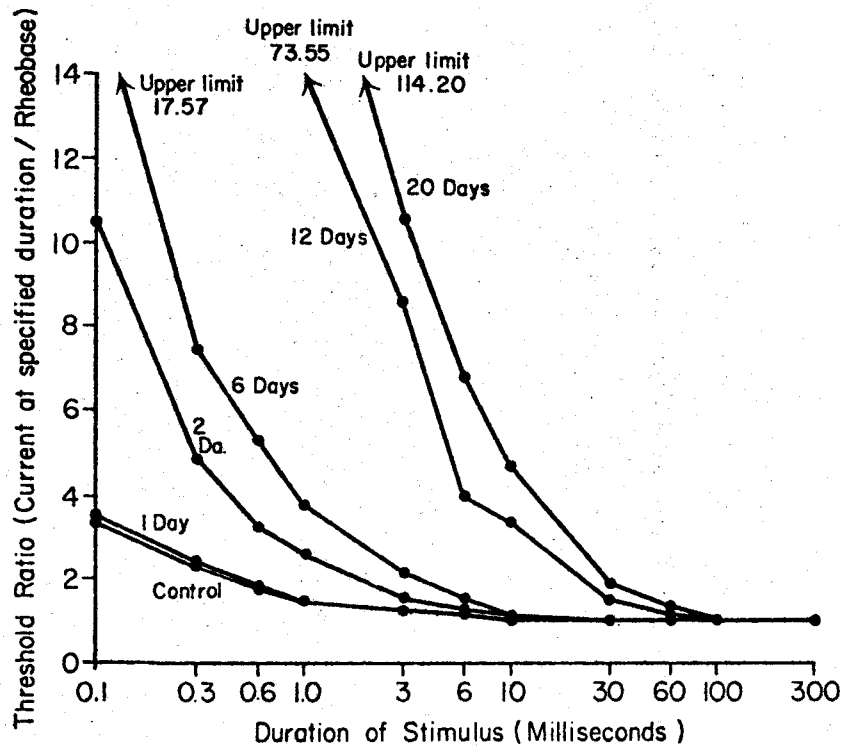
**Figure 8**





**Strength Duration Curves in Local Tetanus**  
Each point represents the mean from the observation on 8 animals

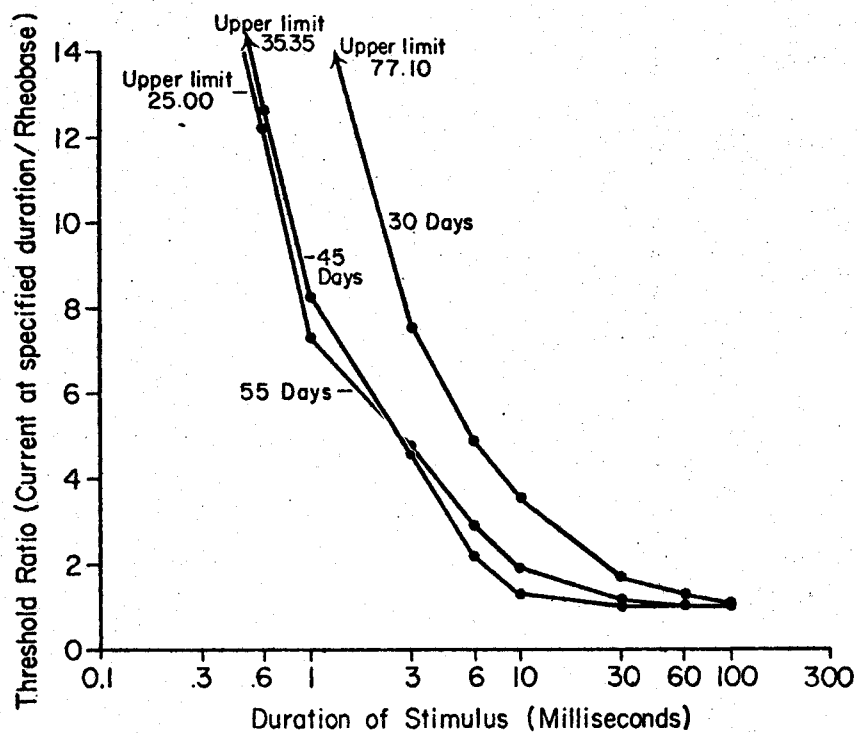
Figure 9



### Strength Duration Curves in Chronic Denervation

Each point on the curve represents the mean from the observations on 6 animals

Figure 10



### Strength Duration Curves in Chronic Denervation

Each point represents the mean from the observation on 6 animals

Figure 11

shown in Fig. 8, 9, and 10, 11. Figures 9 and 11 are the continuation of Figures 8 and 10.

### Chronaxie

Chronaxie values, in all cases, were determined from the individually plotted strength-duration curves. The values so obtained from each individual curve were averaged on each day of examination and the standard deviations were computed. Table IV represents the chronaxie values in local tetanus and chronic denervation respectively. The graphs of the values are shown in Fig. 12.

### Galvanic-Tetanus Ratio

The Galvanic-Tetanus Ratio (cathodal) was obtained by dividing the current required for tetanic type of contraction by the current required for simple twitch (Rheobase). Table V represents such data in local tetanus and chronic denervation respectively. The data are plotted in Fig. 13.

### Responses to repetitive stimulation

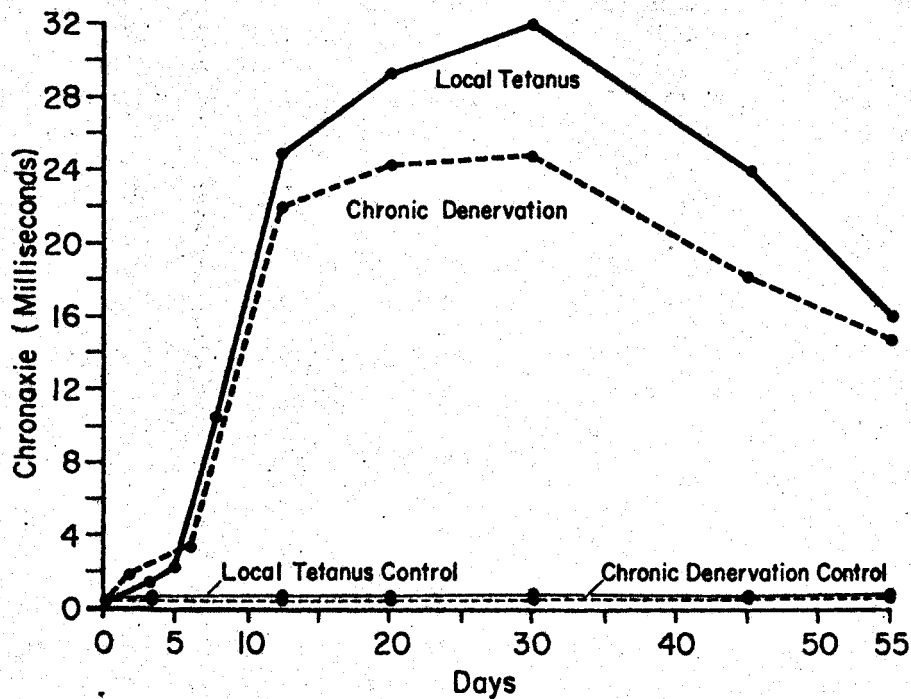
The actual current values required for tetanic contraction with each of the three different frequencies (91, 166 and 500 cycles per second) were converted into threshold ratios. The averages and their standard deviations were computed for each group on each day of the examination. Table VI represents such data for local tetanus and chronic denervation. They are plotted in Fig. 14 and 15 respectively.

Tables VII and VIII summarize electromyographic and

TABLE IV

## CHRONAXIE IN LOCAL TETANUS AND CHRONIC DENERVATION

Days after toxin injection or nerve section	Local Tetanus (8 animals)		Chronic Denervation (6 animals)	
	Avg.	S.D.	Avg.	S.D.
1	0.37	± .21	0.44	± .08
2	1.41	± .45	1.8	± .60
5	2.15	± .47		
6			3.31	± .82
8	10.41	± 1.91		
12	25.32	± 3.51	22.2	± 6.07
20	29.6	± 3.16	24.5	± 3.74
30	32.1	± 10.52	24.8	± 4.35
45	24.12	± 4.23	18.33	± 4.21
55	16.51	± 3.32	16.21	± 3.89
Control	0.35	± .08	0.37	± .10



### Chronaxie in Local Tetanus and Chronic Denervation

Each point on the curve represents the mean from observation on 8 animals (Local Tetanus) and on 6 animals (Chronic Denervation).

Figure 12

## GALVANIC TETANUS RATIO IN LOCAL TETANUS AND CHRONIC DENERVATION

Days after toxin injection or nerve section	Local Tetanus (8 animals)		Chronic Denervation ( 6 animals)	
	Avg.	S.D.	Avg.	S.D.
1	3.32	± .24	3.22	± .39
2			3.45	± .47
3	3.21	± .41		
5	3.40	± .40		
6			6.01	± .48
8	5.18	± .75		
12	5.77	± .87	6.31	± 1.34
20	6.07	± 2.99	6.40	± .65
30	5.82	± .96	6.02	± .61
45	3.51	± .42	4.82	± .52
55			4.13	± .43
Control	3.33	± .25	3.38	± .41

TABLE VI

## REPETITIVE STIMULATION IN LOCAL TETANUS (8 ANIMALS)

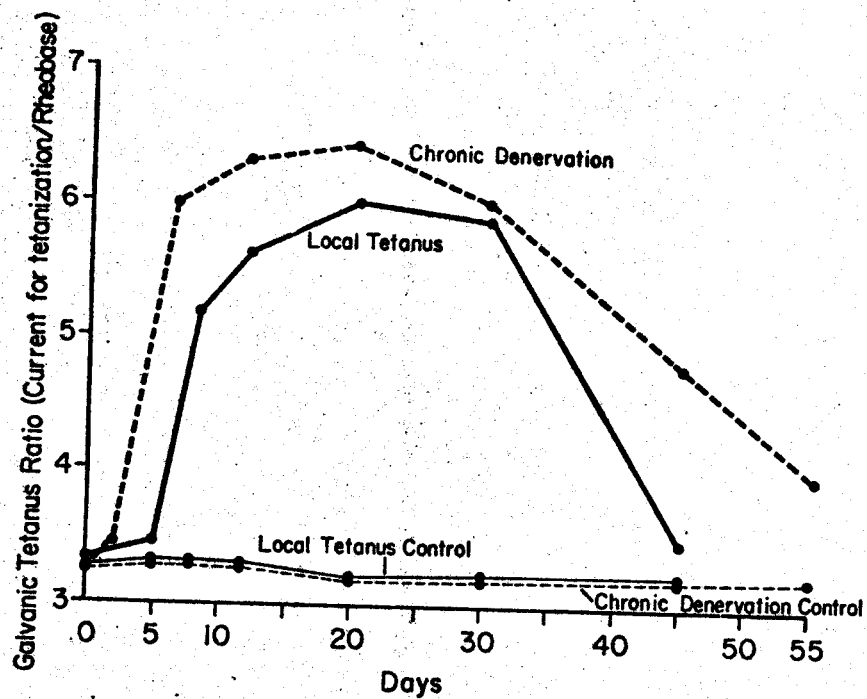
Frequency (CPS)	Control Side	<u>Affected Side</u>					
		<u>Days After the Injection of T. Toxin</u>					
		<u>1 Day</u>	<u>3 Days</u>	<u>5 Days</u>	<u>8 Days</u>	<u>12 Days</u>	
		<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	
91	1.58+.37	1.46+.35	1.63+.43	1.45+.16	9.15+1.30	32.82+2.15	
166	1.48+.22	1.47+.26	1.69+.42	1.72+.19	8.14+1.18	27.13+9.16	
500	1.78+.26	1.73+.28	1.98+.51	1.74+.28	8.16+1.55	21.43+7.74	
		<u>20 Days</u>	<u>30 Days</u>	<u>45 Days</u>	<u>55 Days</u>		
		<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>		
		15.16+361	10.79+343	2.91+.28	2.61+.33		
		15.45+1.52	10.55+368	3.02+.37	2.46+.34		
		13.66+394	9.06+608	3.90+.38	2.82+.29		

## REPETITIVE STIMULATION IN DENERVATION (6 ANIMALS)

Frequency (CPS)	Control Side	<u>Denervated Side</u>				
		<u>Days After Denervation</u>				
		<u>1 Day</u>	<u>2 Days</u>	<u>6 Days</u>	<u>12 Days</u>	
		<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	
91	1.55+.28	1.53+.31	3.94+.52	7.28+3.08	27.4+24.97	
166	1.51+.28	1.51+.35	3.87+.44	8.31+1.61	24.1+8.68	
500	1.76+.31	1.87+.29	8.06+.75	11.47+6.35	14.4+4.02	
		<u>20 Days</u>	<u>30 Days</u>	<u>45 Days</u>	<u>55 Days</u>	
		<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	<u>Avg.S.D.</u>	
		40.12+570	20.63+353	19.5+6.24	10.36+3.94	
		38.34+549	28.35+372	16.5+5.87	8.84+3.33	
		24.62+558	18.42+337	11.2+4.33	9.62+3.49	

S.D. = Standard Deviation

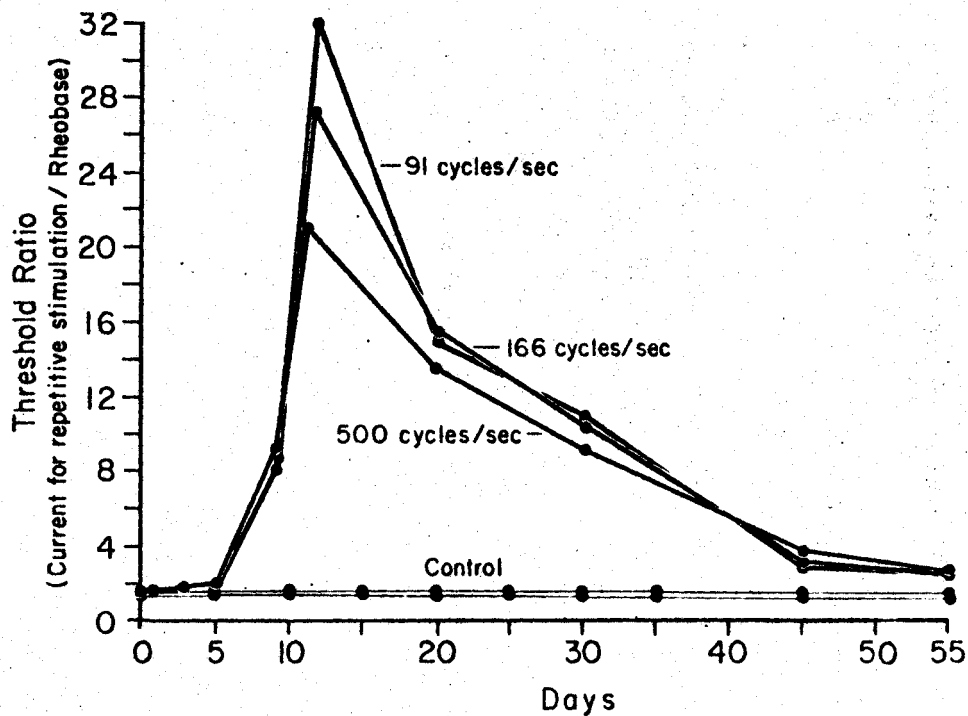




### Galvanic Tetanus Ratio in Local Tetanus and Chronic Denervation

Each point on the curve represents the mean from the observations on 8 animals (Local Tetanus) and on 6 animals (Chronic Denervation).

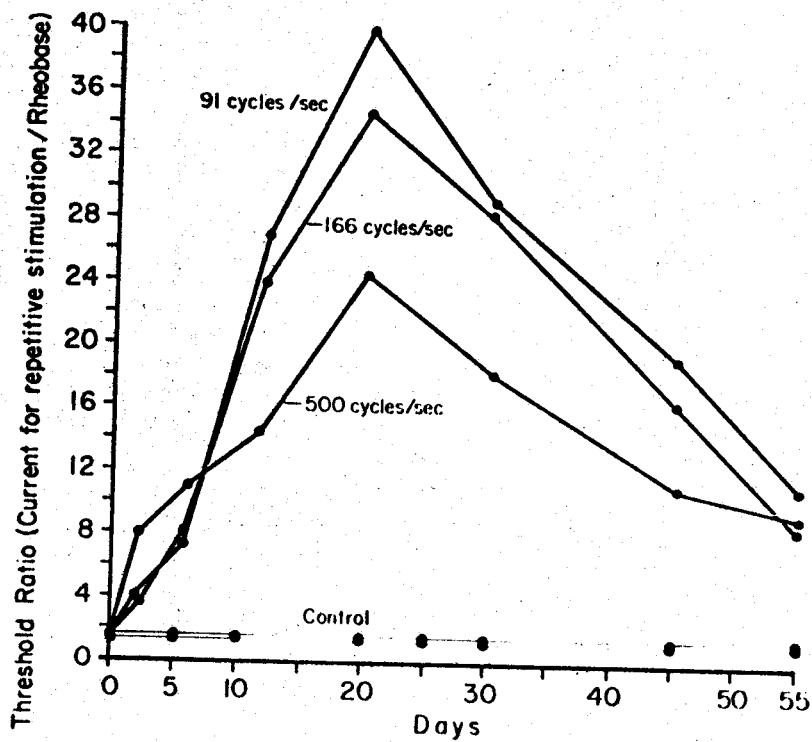
Figure 13



### Repetitive Stimulation in Local Tetanus

Each point represents the mean from the observation on 8 animals

Figure 14



**Repetitive Stimulation in Chronic Denervation**  
Each point represents the mean from the observation on 6 animals

**Figure 15**

TABLE VII

## SUMMARY OF ELECTRODIAGNOSTIC FINDINGS

Experimental Procedure	Control Side	Local Tetanus	Chronic Denervation
Rheobase	No change with time	Initial rise for 4-5 days followed by a significant decrease	Initial rise followed by a significant decrease
Strength-Duration Curves	Rectangular hyperbola. No change with time	Significant upward trend as the local tetanus develops. Discontinuities seen. High current values are necessary for stimulation at lower stimulus duration.	Significant upward trend in the curves with time. High current values are necessary for stimulation at lower stimulus duration.
Chronaxie	No change with time	No significant change in first 5 days. Rapid and continuing increase with progressive local tetanus.	No significant change in the first 5 days. Rapid and continued increase with progressive denervation.
Galvanic-Tetanus Ratio	Within normal limits; no change with time	Rapid increase with time	Rapid increase with time
Repetitive Stimulation	Normal pattern, within normal limits. No change with time.	Marked increase in threshold and change in pattern.	Marked increase in threshold and change in pattern.

## SUMMARY OF ELECTROMYOGRAPHIC FINDINGS

Experimental Procedure	Control Side	Local Tetanus	Chronic Denervation
Potentials at rest	Electrical silence	Electrical silence very difficult to obtain, in early stages	Electrical silence for first 4-5 days. Spontaneous fibrillation potentials in the following period.
Potentials due to movement	Normal motor units mostly biphasic	Intense electrical activity consisting mostly of motor units for 4-5 days. Then a mixture of polyphasic potentials and positive sharp waves - 6-10 days. Absence of motor units and complete replacement by spontaneous fibrillation potentials. Recovery is essentially in the reverse order.	Movements have no effect on the fibrillation potentials.

electrodiagnostic findings.

The response of the muscle to nerve stimulation and to direct excitation

The tibialis anticus muscle of 12 rabbits under local tetanus at different time periods (2-20 days following the injection of tetanus toxin) and of 8 rabbits under chronic denervation (10-20 days after nerve section) formed the subject of this study. Fig. 16, 17, and 18 represent typical records from such stimulation study.

In local tetanus the mechanical response of the muscle to indirect stimulation underwent a progressive diminution and eventually a complete abolition with progress of time. In the muscles tested 3-4 days after the injection of the toxin the isometric twitches to indirect stimulation were reduced to about 60-70% of the normal. Similar testing carried out in other groups of rabbits with 4-5 days old local tetanus gave a response of about 30-40% of the normal. This mechanical response to indirect stimulation was completely abolished in rabbits in which the local tetanus was 6-7 days old. (Fig. 16 a,b,c) On the other hand, the mechanical response to direct stimulation remained essentially the same as the normal throughout this period though the isometric tension was slightly reduced.

The isometric twitches of the muscle in chronic denervation in response to direct excitation were essentially the same as those obtained from the direct excitation of the muscle in advanced

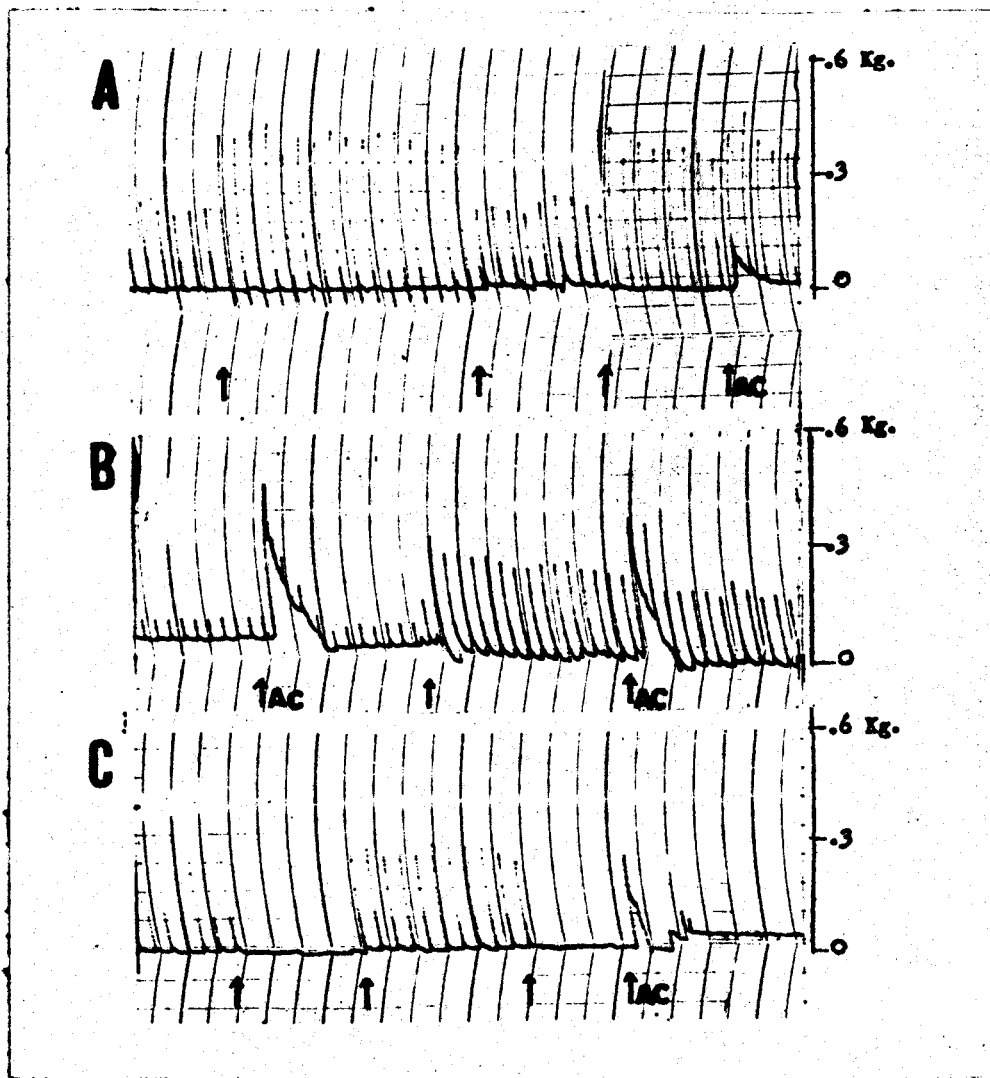


Figure 16

A. Rabbit 2.1 Kg., spinal transection between T<sub>6</sub>-T<sub>12</sub>, isometric contractions at 10 sec. intervals of tibiialis muscle in which tetanus toxin was injected 3 days previously. At first arrow change from maximal motor nerve volleys to maximal direct stimuli. Ac. 15 u/Kg. Acetylcholine, second arrow, third arrow.

B. Rabbit 1.8 Kg. tetanus toxin injected 5 days previously. At first arrow change from maximal motor nerve volleys to direct stimuli. Ac. 15 u/Kg. acetylcholine.

C. Rabbit 2.2 Kg. tetanus toxin injected 7 days previously. At first arrow change from maximal direct volleys to maximal nerve stimuli. Ac. 15 u/Kg. acetylcholine.

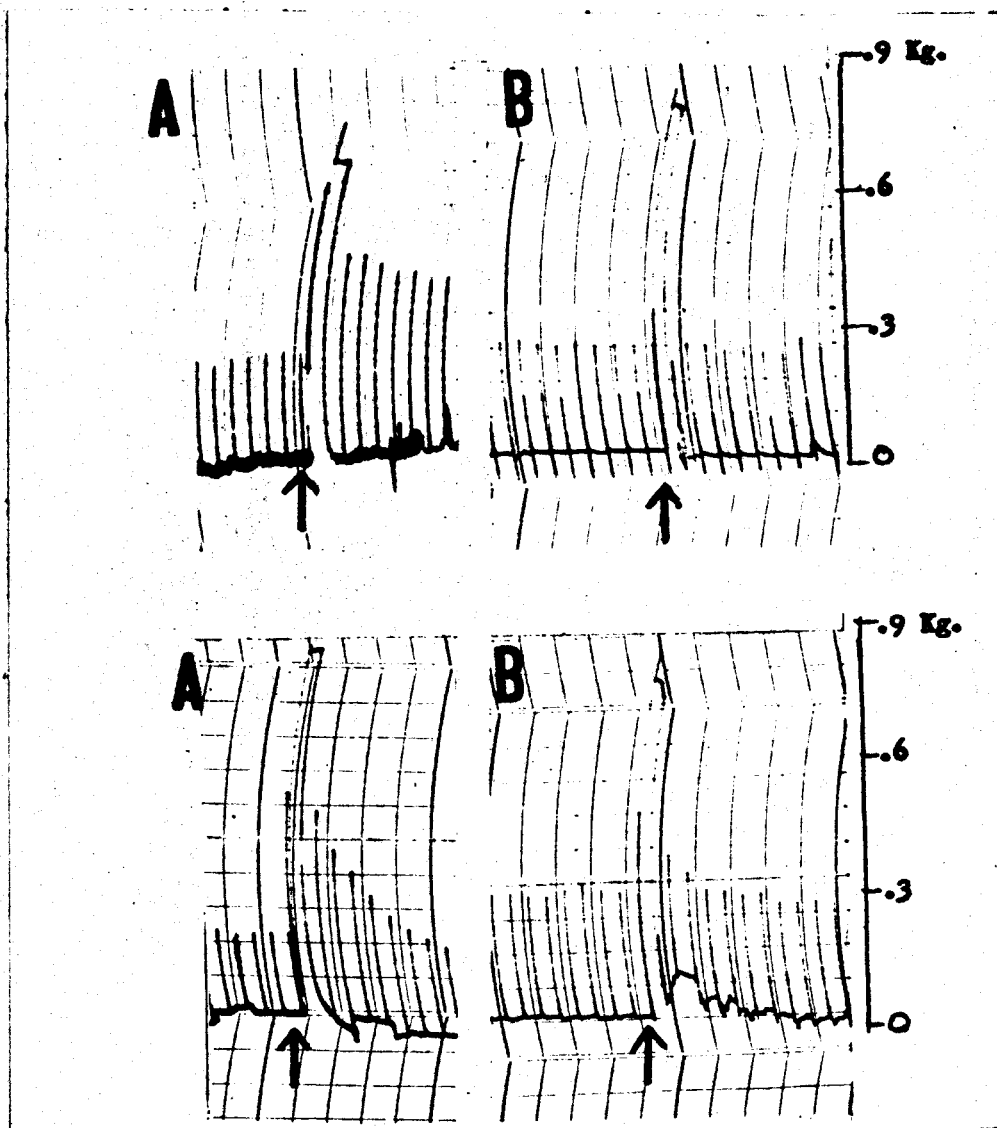


Figure 17

Upper: :

Rabbit 2.0 Kg. Spinal transection between T<sub>6</sub>-T<sub>12</sub>. Isometric contractions of the tibial muscle. A. Normal side. At arrow maximal motor nerve tetanus at 100 per sec. for 10 sec. B. Local tetanus side; tetanus toxin injected 15 days previously. At arrow maximal direct tetanus at 100 per sec. for 10 sec.

Lower:

Rabbit 1.85 Kg. Chronic denervation 13 days previously. A. Normal side. B. Denervated side. Isometric contractions of the tibialis muscle. At arrow maximal muscle tetanus at 100 per sec. for 10 sec.



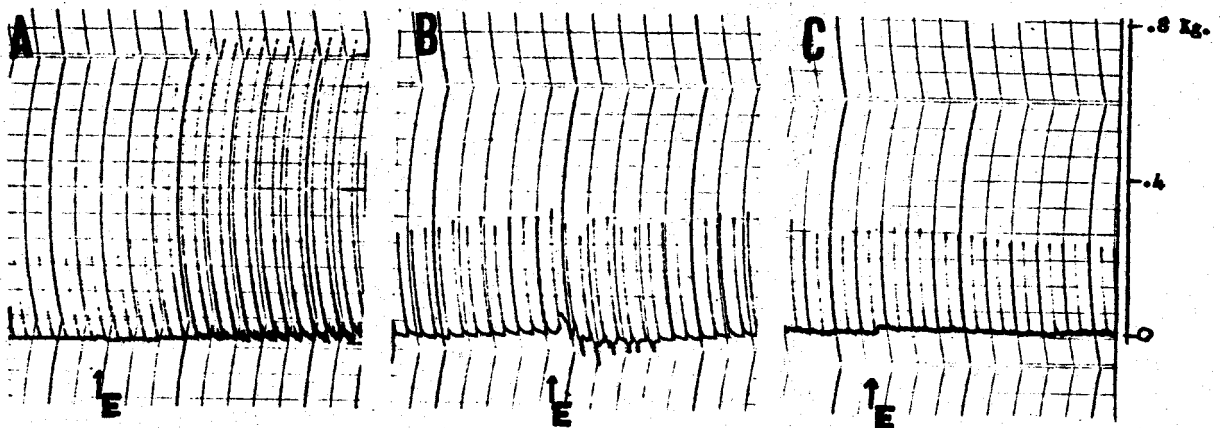


Figure 18

Isometric contractions of the tibialis at 10 sec. intervals to maximal direct volleys.

- A. Rabbit 1.75 Kg., normal, at E, eserine, 25 ug/Kg.
- B. Rabbit 1.9 Kg., tetanus toxin injected 15 days previously. At E, eserine, 25 ug/Kg.
- C. Rabbit 1.7 Kg., denervated 14 days previously. At E, eserine, 25 ug/Kg.

local tetanus. (8-20 days)

The potentiation of the single twitches immediately following a brief period (10-12 seconds) of tetanic stimulation (100 shocks per second) is a constant finding in a normal muscle. However, the muscle in advanced local tetanus (8-20 days) and in chronic denervation (1-2 weeks following nerve section) when similarly stimulated did not show post-tetanic potentiation. (Fig. 17)

#### Effect of Ach and Eserine

When a small dose of Ach (15-20 ug/kg) was administered intraarterially (distant route) to a muscle in early local tetanus an initial quick twitch-like response was obtained which was almost similar to that seen in normal muscle. However, in advanced local tetanus the twitch tension instead of immediately relaxing gradually decreased over a period of 20-30 seconds. (Fig. 16 b) Similar response was also observed in chronically denervated muscle.

When 25 ug/kg of eserine was injected intraarterially into a muscle in advanced local tetanus no increase in the tension to single direct twitches was obtained. Eserine injected in a similar manner into an animal with a muscle in chronic denervation also did not exert any increase in the tension of the muscle. However, the same dose in the opposite normal muscle increased the tension very considerably in both cases. (Fig. 18)

### Responses to pharmacological agents

The results of the electromyographic, electrodiagnostic and electro-stimulation study reported above clearly indicated a close similarity between a muscle in advanced local tetanus and chronic denervation. This was further substantiated by the responses of these two types of muscle to some of the well established pharmacological agents.

Chronically denervated skeletal muscle is known to be 100-100,000 times more excitable to acetyl choline when it is administered by close arterial injection, Brown (1937), Rosenbleuth and Luce (1937), and others. In addition to being supersensitive to Ach, chronically denervated muscle also shows an enhanced responsiveness to certain other agents such as nicotine, choline and tetramethyl ammonium etc. The following agents were selected for this study: Ach, Decamethonium, Succinylcholine, Physostigmine, Tetra ethyl pyrophosphate, Di-isopropyl flubro phosphate, d-Tubocurarine. Tables IX and X summarize the pharmacological findings with these agents.

#### Acetylcholine (Ach)

The immediate response of the muscle in advanced local tetanus (10-20 days) to a small dose of Ach, given arterially is an out-burst of electrical activity greatly in excess of any previous spontaneous activity. (Fig. 19) This phenomenon is very striking when monitored by sound, because the background sound of the fibrillation potentials suddenly jumps to a roar immediately

## CHAPTER IX

## SUMMARY OF PHARMACOLOGICAL FINDINGS

## LOCAL TETANUS

Drugs	Doses, Distant arterial route	Effects on the Fibrillation Potentials
Ach (6)*	0.5 ug/kg 5-10 ug/kg 100 ug/kg	Shower of activity - 5-6 seconds Shower of activity - 25-45 seconds No electrical silence Shower of activity for 20 seconds Sudden electrical silence for 2-3 minutes then back to normal
C10 (5)	0.5 ug/kg 10 ug/kg 50 ug/kg	Shower of activity - 5-6 seconds Shower of activity - 30-50 seconds Shower of activity - 25 seconds Sudden electrical silence for 6-7 minutes
Succinyl choline (3)	1 ug/kg 50 ug/kg	Same as in C10
TEPP (4)	0.1 mg/kg 0.2 mg/kg	No increased shower of activity Occasional bursts of potentials in the preinjection background activity
DFP (4)	0.1 mg/kg 0.3 mg/kg	Same as in TEPP
Physo- stigmine (4)	.5 mg/kg 1. mg/kg	No shower of activity Twitchings in the innervated muscle
d-TC (6)	10-100 ug/kg 200 ug/kg	No shower of activity. No decrease in activity No shower of activity. No decrease in activity
(2)	500-1000 ug/kg	Decrease in frequency and amplitude, No electrical silence.

\* Numbers in the brackets are numbers of animals used.

## SUMMARY OF PHARMACOLOGICAL FINDINGS

## CHRONIC DENERVATION

Drugs	Doses, Distant intra arterial route	Effects on the Fibrillation Potentials
Ach (4)*	0.5 ug/kg 5-10 ug/kg 100 ug/kg	Shower of activity - 4-6 seconds Shower of activity - 30-45 seconds No electrical silence Shower of activity - 20-25 seconds Sudden electrical silence 2-2.5 minutes then back to normal
C <sub>10</sub> (3)	0.5 ug/kg 10 ug/kg 50 ug/kg	Shower of activity - 5-7 seconds Shower of activity - 32-50 seconds Shower of activity - 20-30 seconds Sudden electrical silence for 6-8 minutes, then back to normal
Succinyl choline (3)	1 ug/kg 10 ug/kg 50 ug/kg	Same as in C <sub>10</sub>
TEPP (3)	0.1 mg/kg 0.2 mg/kg	No shower of activity Occasional additional bursts of fibril- lation in the background fibrillation activity
DEP (3)	0.3 mg/kg	Essentially same as in TEPP
Physo- stigmine (4)	.5 mg/kg 1.0 mg/kg	No shower of activity Considerable twitchings in the innerva- ted muscle
B-TC (4)	10-100 ug/kg 200 ug/kg (2) 500-1000 ug/kg	No shower of activity No shower of activity Decrease in frequency and amplitude No electrical silence

\* Numbers in the brackets are the numbers of animals used.

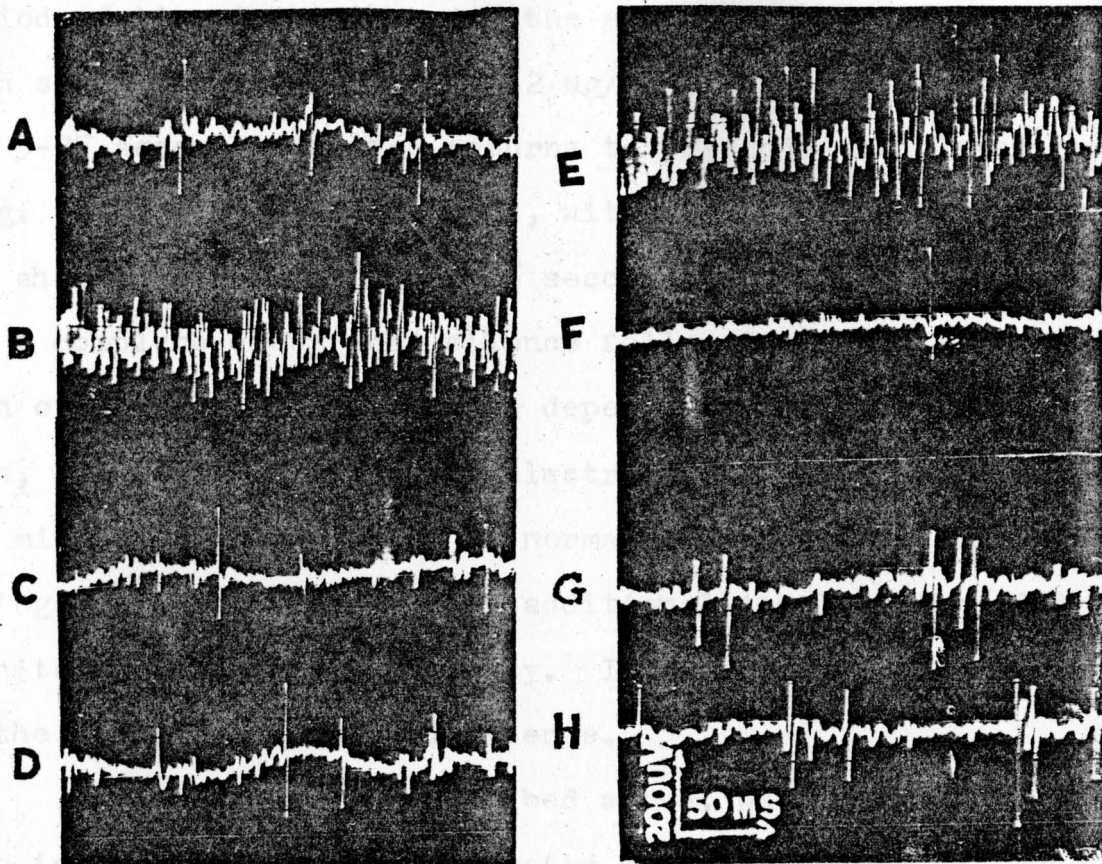


Figure 19

Rabbit 1.9 Kg. Effect of ACh on the spontaneous fibrillation potentials of the tibialis in local tetanus. Injection of tetanus toxin 15 days previously.

- A. Fibrillation potentials.
- B. Increased shower of fibrillation potentials after ACh, 1.0 ug/Kg., i.a.
- C. and D. Back to preinjection level of activity.
- E. Increased activity. ACh, 50 ug/Kg.
- F. Electrical silence.
- G. and H. Fibrillation potentials after 2 minutes.

following the injection of Ach. At the same time the oscilloscope picture shows a shower of fibrillation potentials. (Fig. 19 b) This roar and shower of electrical activity last for a varying period of time depending upon the amount of Ach injected. Thus, with small doses, such as 0.5-2 ug/kg the shower of activity lasts for 5-25 seconds and then returns to pre-injection level.

(Fig. 19 c) On the other hand, with larger doses, (25-50 ug/kg) the shower lasted for about 45 seconds and then stopped suddenly and a complete electrical silence followed. (Fig. 19 f) The duration of the electrical silence depends again, upon the dose of Ach. Thus, with 100 ug/kg Ach the electrical silence lasted for about 2-3 minutes, then returned to normal pre-injection level. (Fig. 19h) During the electrical silence additional Ach injection did not elicit any electrical activity. It only increased the duration of the existing electrical silence.

The phenomena described above, was consistently seen also in the chronically denervated muscle. These two types of muscle, local tetanus and denervation, responded to Ach in a manner indistinguishable from each other. (Fig. 19 and 20)

#### Decamethonium and Succinylcholine

These two compounds which have been described as depolarizing agents gave essentially the same type of response in both local tetanus and denervation as that produced by Ach. The only appreciable difference between these two agents and Ach was in the duration of electrical silence. The threshold dose for eliciting

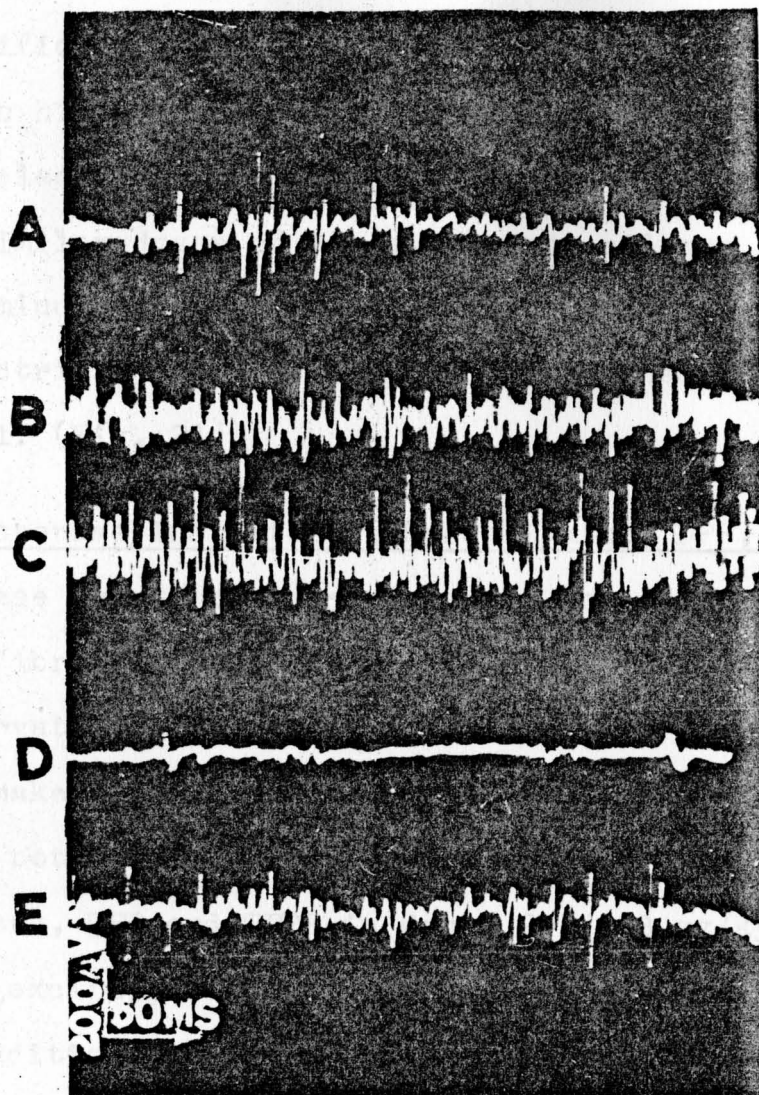


Figure 20

Rabbit 2.1 Kg. Chronic denervation (14 days).

Effect of Ach.

- A. Fibrillation potentials.
- B. Increased shower of fibrillation potentials after Ach, 1.0 ug/Kg., i.v.
- C. Ach, 50 ug/Kg. Increased activity.
- D. Electrical silence.
- E. Fibrillation potentials after 1 1/2 minutes.



the response with decamethonium and succinylcholine (0.5-0.8 ug) was not significantly different from that of Ach (0.3-0.5 ug). However, with higher doses of decamethonium and succinylcholine (50 ug) the electrical silence lasted for about 7-8 minutes. While with similar doses of Ach electrical silence lasted for about 1-1.5 minutes. In every case in which these agents were used the electrical activity eventually returned to the preinjection level. (Fig. 21)

#### Cholinesterase inhibitors (Physostigmine, DFP, and TEPP)

These agents by themselves did not affect the background spontaneous fibrillation potentials, either in local tetanus or chronic denervation. Physostigmine in doses of 0.5-1 mg/kg did not seem to make any difference in the frequency and amplitude of fibrillation potentials. However, the organo-phosphorous anticholinesterases, DFP and TEPP in doses 0.1-0.3 mg/kg appeared to produce some excitatory response in the muscle as evidenced by occasional additional bursts of fibrillation-like potentials. At no time however, was there such a typical shower of activity as was obtained with Ach, decamethonium and succinylcholine.

#### Effect of Ach after Cholinesterase inhibitors

Administration of a threshold dose (0.5 ug) of Ach following the cholinesterase inhibitors (Physostigmine, DFP and TEPP) gave essentially the same type of response as that produced by Ach alone. That is the shower of electrical activity lasted for

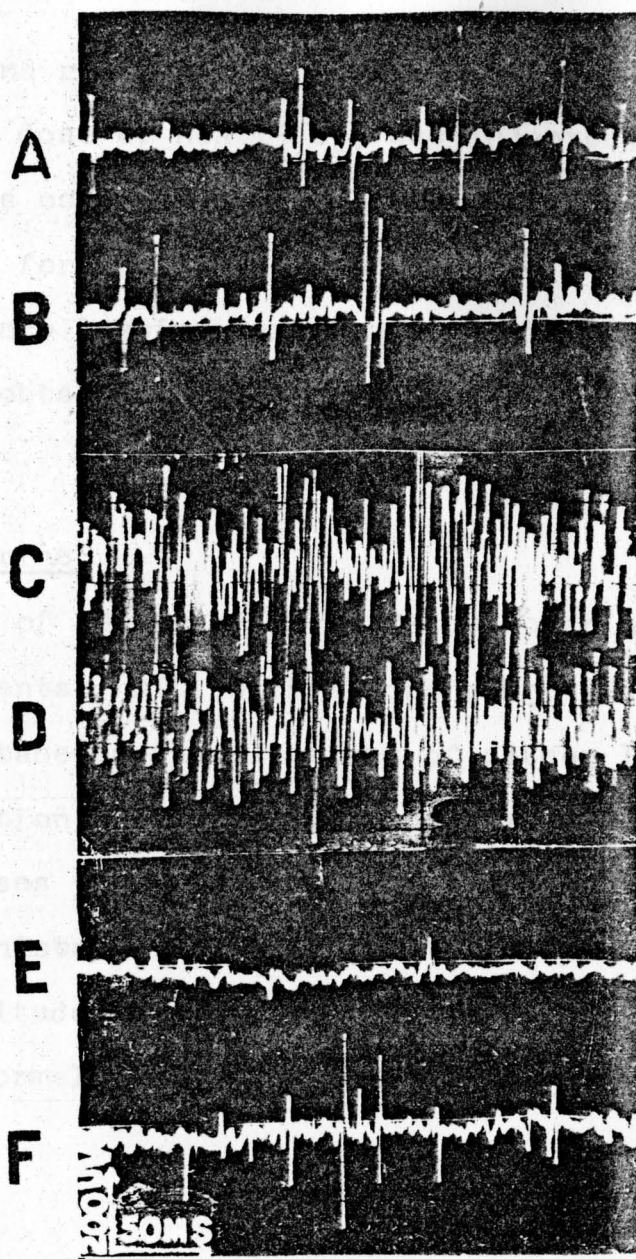


Figure 21

Rabbit 1.85 Kg. Effect of decamethonium on the spontaneous fibrillation potentials of the tibialis in local tetanus. Injection of tetanus toxin 14 days previously.

- A. and B. Spontaneous fibrillation potentials.
- C. After 1.0 ug/Kg. of decamethonium intraarterially. Immediate increase in activity ("shower").
- D. After 50 ug/Kg. of decamethonium intraarterially. Immediate increase in activity ("shower").
- E. Electrical silence.
- F. Electric activity returns to preinjection levels after 9 minutes.

a few seconds and rapidly came back to normal. However, when a slightly larger dose of Ach (5 ug) was injected, the duration of the response was considerably longer. The shower of electrical activity lasted for about 30-35 seconds and was followed by an electrical silence lasting for about 2-3 minutes. This response resembled that obtained with a dose of 75-100 ug of Ach alone. (Fig. 22, 23)

#### d-Tubocurarine

Doses of 10-200 ug/kg of d-tubocurarine were given in several experiments. DTC in this dose range did not seem to affect the spontaneous fibrillations of either local tetanus or chronic denervation. However, in a few experiments where relatively large doses 500-1000 ug/kg of DTC (3-5 times the paralytic dose) were administered under artificial respiration, the frequency and amplitude of fibrillation potentials decreased and did not return to normal preinjection level even after two hours. (Fig. 24)

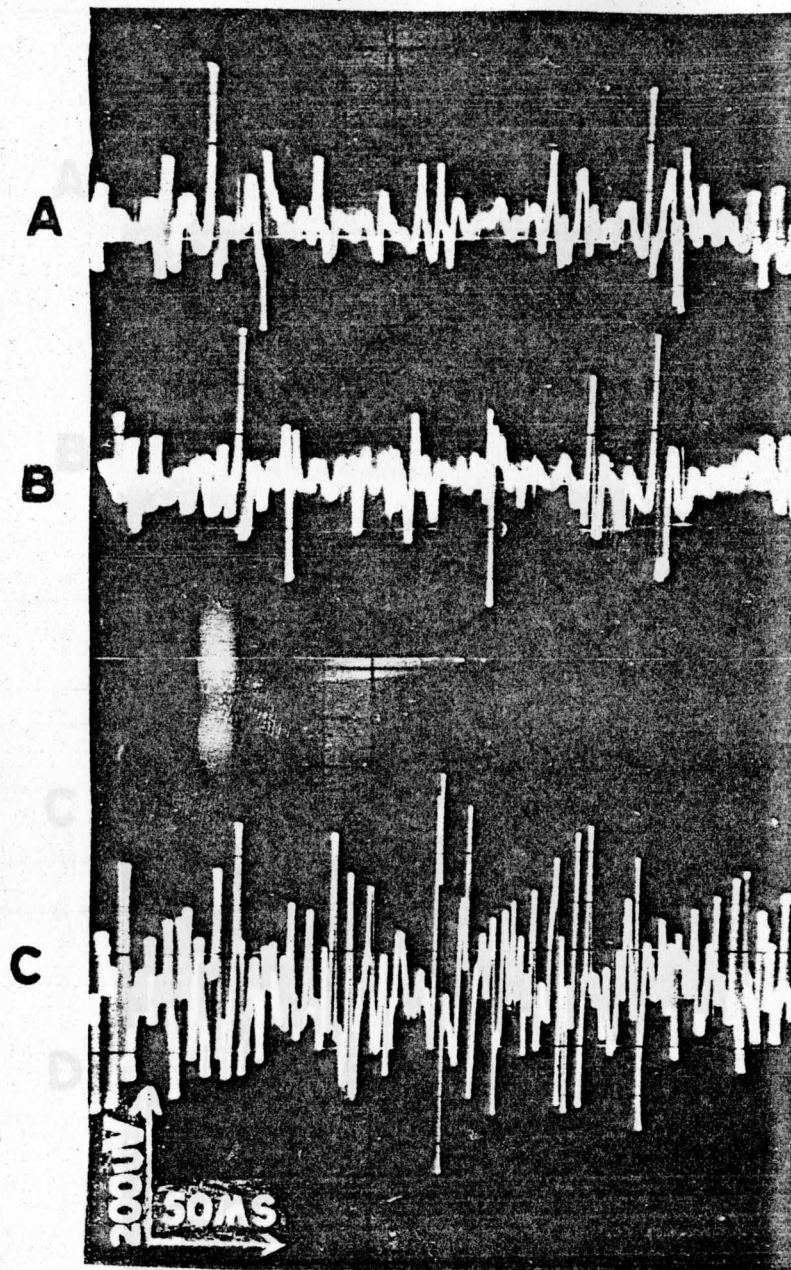


Figure 22

Rabbit 1.95 Kg. Effect of DFP on the spontaneous fibrillation potentials of tibialis in local tetanus. Injection of tetanus toxin 16 days previously.

- A. Spontaneous fibrillation potentials.
- B. After 0.2 mg/Kg. of DFP intraarterially.
- C. After 1.0 u/Kg. of Ach intraarterially.



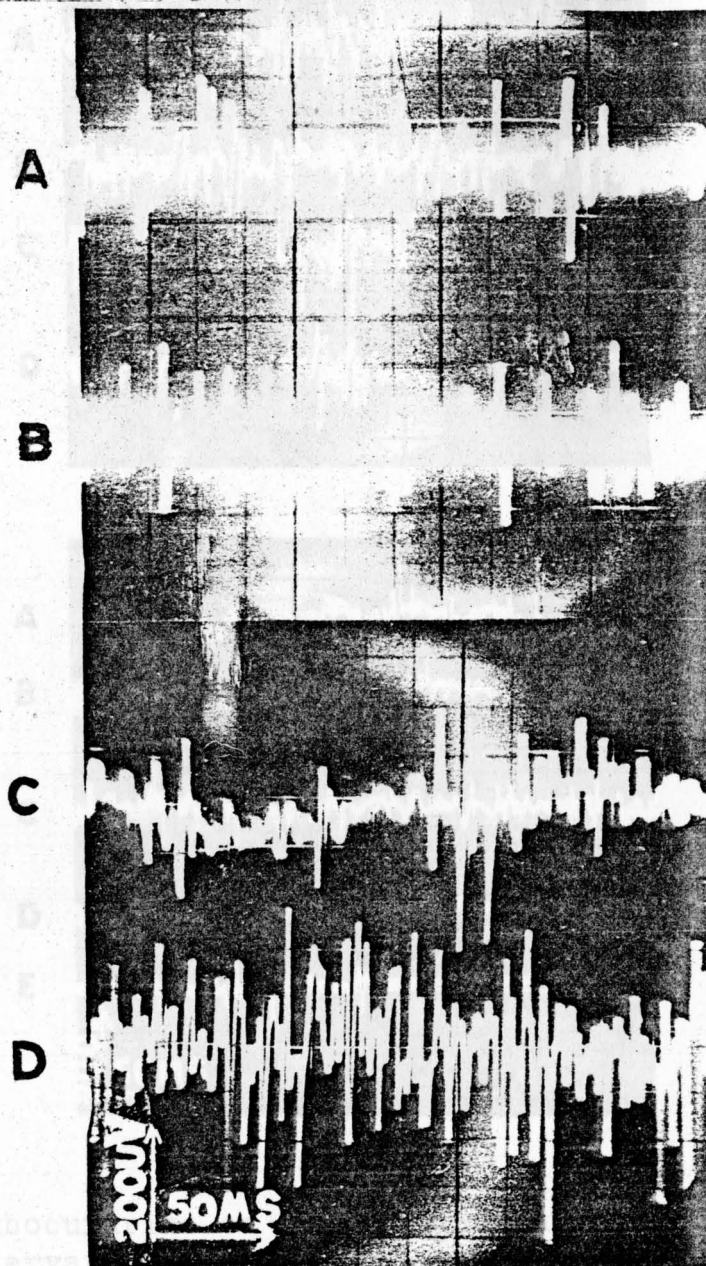


Figure 23

Rabbit 2.1 Kg. Effect of TEPP on the spontaneous fibrillation potentials of tibialis in local tetanus. Injection of tetanus toxin 14 days previously.

- A. Spontaneous fibrillation potentials.
- B. After 0.1 mg/Kg. TEPP intraarterially.
- C. Fibrillation potentials after 7 minutes.
- D. After 1.0 ug/Kg. of Ach intraarterially.

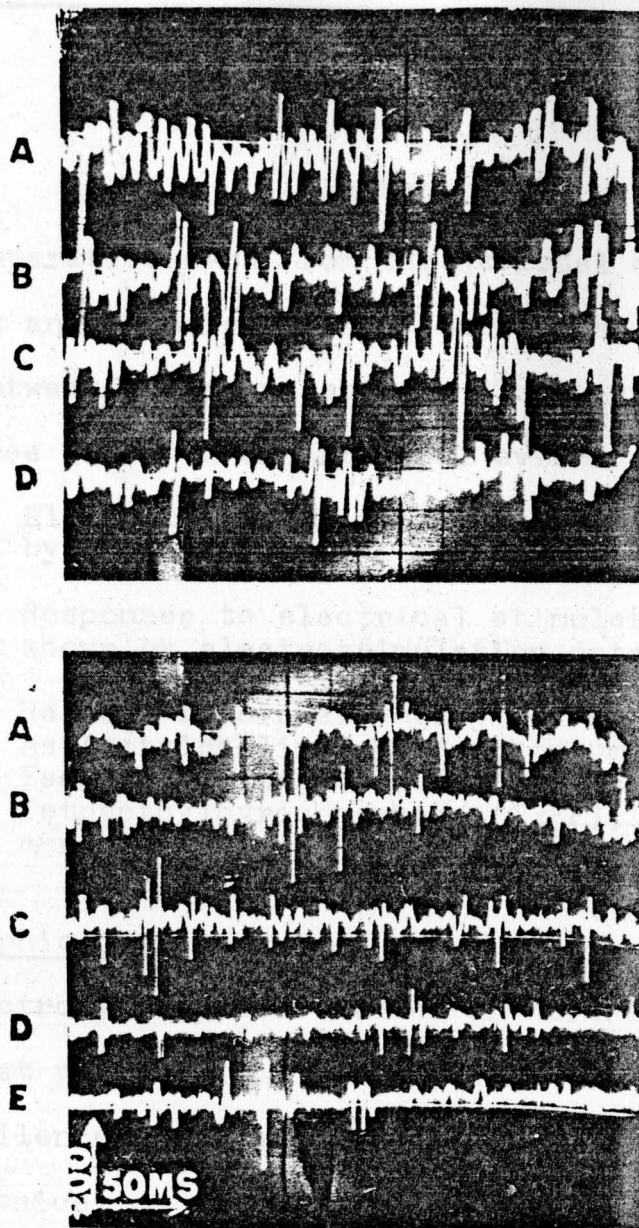


Figure 24

Effect of d-tubocurarine on the spontaneous fibrillation potentials in chronic denervation and in local tetanus. Artificial respiration. Upper- Rabbit, 1.85 Kg., denervated 14 days previously.

A. Spontaneous fibrillations. B. After d-Tc, 50 ug/Kg., intra-arterially. C. 20 minutes after d-Tc, 100 ug/Kg., intraarterially. D. 5 minutes following d-Tc, 350 ug/Kg., intraarterially.

Lower- Rabbit, 2.0 Kg. Local tetanus produced 17 days previously.

A. Spontaneous fibrillations. B. After d-Tc, 50 ug/Kg., intra-arterially. C. After d-Tc, 200 ug/Kg., intraarterially. D. After 5 minutes following d-Tc, 350 ug/Kg., intraarterially. E. 2 hours later.

## CHAPTER IV

### DISCUSSION

Comparative data from the skeletal muscle in progressive local tetanus and chronic denervation indicate a remarkable similarity between these two states. This is established by the following three lines of experimental evidences:

- a. Electrical activity in the muscle - as shown by EMG data
- b. Responses to electrical stimulation - as shown by electrostimulation data
- c. Responses to pharmacological agents - such as Acetylcholine, Decamethonium, Succinylcholine, Eserine, Diisopropyl flurophosphate, (DFP) Tetraethylpyrophosphate (TEPP), and d-Turbo-curarine.

#### Electromyographic data

Electromyographic features of normal skeletal muscle of the rabbit just prior to the manifestation of local tetanus are "electrical silence" at rest and production of motor unit potentials on mechanical or reflex movement. The amplitude and frequency of these potentials depend upon the degree of contraction. Thus, with moderate to high degree of contraction a large number of units are recruited and the electromyogram shows a partial or complete "interference pattern", suggestive of a high degree of electrical activity in the muscle. A similar kind of electrical activity is manifested in the early stages (2-5 days) of local

tetanus and is concomitant with the hyper-reflexive bouts. This apparently spontaneous and unremitting electrical discharge in local tetanus depends upon the spinal segments supplying the affected muscle because sectioning of the motor nerve or the administration of central depressant agents (general anesthetics) block this heightened response. The sensitivity of the muscle in early local tetanus also appears to be increased. This is evident from the observation that even the slightest stimulus such as touch, sound etc., which usually has no effect on the normal muscle, evokes a tremendous shower of electrical activity. This observation confirms the reports of previous investigators, Ranson (1928), Acheson et al (1942), Purdrup (1946). Wright and his co-workers (1952-1954).

The mechanism by which this heightened electrical output is brought about has been a subject of considerable study in recent years. Eccles and his collaborators (1957), Curtis (1959), Wilson et al (1960) have shown that the heightened electrical discharge which is ultimately manifested as high frequency motor unit discharge, originates in the spinal nuclei. They suggest that the tetanus toxin primarily acts at the synaptic regions of the anterior horn cells. The exact mechanism by which the toxin acts, is not yet clear. However, they postulate that the toxin, in some way removes the normal inhibitory influences on the spinal motor neurons. In this respect the tetanus toxin mimics strychnine.

The mechanism of the action of strychnine has been



studied in considerable detail, in recent years. Bradley et al (1953) noted that strychnine in small doses greatly diminishes the inhibition produced by an inhibiting volley, without significantly affecting the testing monosynaptic reflex. Strychnine also diminishes the inhibitory post synaptic potentials (IPSP) produced by any type of inhibitory action on motor neurons with no effect on excitatory post synaptic potentials (EPSP). Further, a possibility that strychnine might depress the inhibitory action by blocking the excitatory synaptic action on the inhibitory neurons themselves, interpolated in the inhibitory pathway, has been excluded on the grounds that no diminution in the electrical responses is obtained from the surface of the spinal cord by the activity of these inhibitory neurons, Eccles (1957). The latter investigator inclines to believe that strychnine acts competitively with the inhibitory transmitter for the receptor patches of the inhibitory subsynaptic membrane. In other words, Eccles regards strychnine as the "curare" of the inhibitory synapse.

The mechanism by which tetanus toxin removes such inhibition is postulated as follows: (a) the tetanus toxin may prevent the release of so-called inhibitory transmitter substance in a manner similar to that of botulinum toxin, Brooks (1956), (b) like strychnine, tetanus toxin may become attached to the subsynaptic inhibitory areas on the motor neuronal membrane and prevent the action thereon, of the inhibitory transmitter (Brooks, Curtis and Eccles, 1957).

In general, the early clinical effects produced by the action of strychnine and tetanus toxin are very similar, and on this account Sherrington (1906) suggested that these two substances act similarly on the central nervous system. Recent experimental work has confirmed this, (Brooks et al (1955) and Curtis (1959)).

Thus it appears that the enhanced electrical output in the muscle in the initial stages of local tetanus has a central origin. However, with the progress of local tetanus (5-10 days) the electrical activity of the muscle shows a different pattern: the initial hyperreflexia disappears, the number of motor unit potentials is considerably decreased and in their place a large number of polyphasic motor unit potentials, positive sharp waves (V-waves) and occasional fibrillation potentials are seen. This appears to be a transitional stage where the nervous activity reaching the muscle is impaired. For it has been documented (Jasper and Ballem, 1949, Richardson, 1951, Marinacci, 1959) that the manifestation of such polyphasic potentials and V-waves are the signs of partial nerve-muscle connection which usually occurs during the stages of nerve regeneration and degeneration. The nature of these positive sharp waves, is not well understood, but they are found to occur in a situation where the trophic control is still intact while the excitable sarcolemmal mechanism is no longer under the control of motor nerves. Thus these EMG features suggest that a gradual neuromuscular degeneration is under way. In contrast to this gradual process, the neuromuscular degenera-

tion in experimental nerve section is sudden and hence no polyphasic and positive sharp waves are seen in the initial stages.

With further progress of local tetanus (10-35 days) the electromyogram consists entirely of spontaneous fibrillation potentials. These are independent of central control, since their rate of discharge is unaffected by nerve sectioning or by the administration of central depressant agents. These fibrillation potentials have the same parameters of amplitude, duration, form, and sound as those of experimental denervation, and cannot be distinguished one from the other.

Fibrillations have been known since 1851 when Schiff demonstrated them in the dog's denervated tongue. Since then a large number of investigators have described and studied them in great detail in various species, Langley and Kato (1915), Hines and Knowlton (1933), Brown (1937), Rosenbleuth and Luco (1937), Denny-Brown and Pennybaker (1939), Tower (1939), Eccles (1941), Hayes and Woolsey (1942), Luco and Eyzaguirre (1955), Li et al (1959), Li (1960) and others.

The origin of fibrillation potentials has been a subject of considerable study. Hayes and Woolsey (1942) held the view that the fibrillation potentials of the denervated muscle were initiated at the end-plate region only, because such fibrillatory activity could not be observed in a portion of the muscle fibre devoid of the end-plate zones. This view is no longer held. Li et al (1957) have shown by means of intracellular electrodes that

fibrillation potentials can occur in areas other than the end plate zones of denervated muscle. This has been confirmed by Li and his coworkers (1959) on embryonic tissue culture explants. Muscle fibres in tissue culture also show spontaneous rhythmic contractions resembling in many respects the fibrillatory movements observed in denervated skeletal muscle, Pogogeff (1946), Szepsenwol (1946), Li et al (1959) and Li (1960). Li and his coworkers (1959) and Li (1960) report that there is a remarkable resemblance between the spontaneous rhythmic spike potentials from the chick embryonic skeletal muscle grown in tissue culture and the fibrillation potentials from denervated mammalian skeletal muscle. They suggest that the mechanisms for the production of these potentials are perhaps basically the same, since the explanted muscle in vitro gives rise to new growth of muscle which matures and shows all the histological features of normal adult muscle with the exception of motor end plate areas.

Further, the questions of whether the spontaneous rhythmic fibrillation potentials originate from a specific focal area in the muscle fibre and also whether there is any difference in the membrane properties of intact and denervated muscles, have been investigated by Li. Based on intracellular recording techniques on denervated rat muscle and chick embryonic tissue cultures he reports that these cells show a rhythmic oscillation of the membrane potential and the spontaneous fibrillation spikes originate when the oscillating membrane potential develops a critical

level of depolarization. His work also indicates that no pre-synaptic activity is required for the initiation of rhythmic fibrillation potentials. And, since no neural elements or end plates were found in the cultured muscle the spontaneous spikes must originate at some point along the muscle fibre itself.

### Electrodiagnostic studies

The comparative results from this part of the work also establishes a close similarity between the skeletal muscle in advanced local tetanus and denervation. In discussing the results of each electrodiagnostic procedure the following points may be emphasized.

#### Rheobase

There is a slight initial rise in the rheobase value both in local tetanus and denervation (1-5 days). Between 6-12 days the rheobase values show a sharp decrease in both denervation and local tetanus. The minimum value reached is about 25% of the normal. The muscle appears to be irritable during this period. Rheobase then gradually returns towards normal as reinnervation proceeds. Pollack et al (1945) observed essentially the same pattern as regards the initial rise and a later fall in rheobase in experimental denervation in cats. Recently, Eudema et al (1960) recorded a similar fall in rheobase one week following denervation in rabbits. Ritchie's results (1954) in man, however, vary from this observation. Wynn-Parry (1953) states that

rheobase alone is of little significant value in clinical prognosis. It is however, agreed that in long term denervation, when fibrosis sets in, the rheobase value is likely to rise several times above the normal value and finally the muscle becomes inexcitable.

### Chronaxie

The average chronaxie value of muscle in local tetanus and chronic denervation shows a significant rise from a normal average value of 0.38 milliseconds to about 32 milliseconds, in the course of 12-30 days after the injection of the toxin. A concomitant rise (from 0.38 -26 milliseconds) also occurs in chronic denervation, during the same period. This again, establishes a close similarity between these two phenomena. Further, the time course of recovery of the muscle in the two states goes hand in hand. This is obvious by the reestablishment of the electrical constants of the muscle towards normal. Following a study of many hundreds of strength duration curves Ritchie (1954) concluded that normal chronaxie values usually lie between 0.15-0.8 milliseconds. Bauwens (1950) quoted a value ranging between 0.1-1.0 milliseconds while Pollock's (1945) and Fudem's (1960) average values are lower than 0.5 milliseconds. The normal average values obtained in this investigation are within the limits reported by all the previous workers. As the local tetanus proceeds (12-30 days) the chronaxie values rise by about 70-90 times the normal and return gradually towards normal as the recovery

ensues. It has been well documented that chronaxie of a completely denervated muscle may show a value anywhere between 50-200 times that of the normally innervated muscle. Thus any finding of a chronaxie of over 50 times the normal is an indication of denervation, Harris (1956). The chronaxie values in the present investigation comply with that statement.

### Strength-Duration curves

Strength-Duration curves in both local tetanus and denervation are essentially normal during a period of the first 1-5 days after the starting of the experiment. As the local tetanus and denervation progress, the curves tend to show a steep rise, especially with the shorter durations of stimuli. Discontinuities or kinks appear in the curve. In a normally innervated muscle the intensity of current for minimal contraction is the same over a wide range of pulse durations. This is considered to be due to the great excitability of nerve which has a low chronaxie. When the muscle is stimulated alone, as in denervated muscle, the curve is no longer a horizontal line but rises steeply and no response is elicited at shorter pulse durations. This is due to a much longer excitability of muscle compared to that of nerve. During a stage of partial degeneration as seen between 5-12 days and during the regeneration (partial innervation) between 45-55 days, the curve is again broken and shows kinks, thus indicating the participation by the elements of both excitable tissues - nerve and muscle, Adrian (1916), Pollock et al (1945) Fudema (1960)

### Galvanic-tetanus ratio

The normal muscle yields an average ratio value of about 3.3. The muscle from both the denervated and local tetanus groups however, show a rapid initial rise up to about 6.1 as the local tetanus and denervation progress and then gradually tend to fall towards normal value which is quite in keeping with the recovery of the muscle. Similar values have been reported in the denervated rabbits, Fudema et al (1960).

### Response to repetitive stimulation

The threshold ratio values for all the three frequencies (91, 165, 500 C.P.S.) remain essentially the same as the normal for the first 5 days after the injection of the toxin. With the progress of local tetanus there is a significant rise in the threshold values for all the three frequencies. These tend to return as the recovery ensues. Chronic denervation also shows a similar trend. These results closely correspond with other electrodiagnostic and electromyographic findings reported in this work.

A perusal of the literature did not show any detailed investigation of this nature, carried out on the muscle in local tetanus. The only workers who made a passing reference to strength-duration curve were Acheson and his coworkers (1942). Acheson's group stated that there was no change in the strength-duration curves of the muscle in local tetanus. It is not surprising that these investigators missed this important electro-



physiological finding, since, they examined the muscle at an early stage when the neuromyal involvement had not yet set in. A degeneration type of lesion begins to be evident only 7-12 days following the injection of tetanus toxin and no electrophysiological changes having any significant bearing on this aspect of neuromuscular physiology are manifested until after about a week following the injection of the toxin.

#### Response of muscle to nerve stimulation and direct excitation

When a muscle in early local tetanus (3-4 days) is stimulated indirectly through its motor nerve the isometric twitches are reduced to about 60-70% of the normal. This response progressively decreases with time and eventually is completely abolished (6-8 days). However, when the muscle is stimulated directly, the nature of the twitch remains essentially the same as the normal but the tension is slightly reduced. Chronically denervated muscle when stimulated directly elicits essentially the same type of response as obtained in advanced local tetanus.

Potentiation of the twitches following a short period of tetanic stimulus (100 cycles per second for 10 seconds) is a constant finding in normal muscle. This is absent in the muscle in advanced local tetanus and in chronic denervation as well. These two observations indicate that the muscle in local tetanus and denervation react in a similar manner to the electrical stimulus.

### Effect of Ach

When a distant arterial injection (Methods and Materials) of 15-25 ug/kg of Ach is made into the arterial supply of a muscle in advanced local tetanus, while it is being stimulated through the motor nerve, an initial quick twitch-like response is obtained. The isometric twitch tension, instead of suddenly relaxing decreases gradually over a period of 10-20 seconds. A similar kind of response is also obtained in a directly stimulated muscle in chronic denervation. The maximum tension reached in the former case is as great as that obtained when the muscle in chronic denervation is being stimulated directly. This again demonstrates a similarity between the muscle in advanced local tetanus and chronic denervation.

### Effect of Eserine

Eserine in a normal muscle gives an increase in twitch tension (sometimes up to 100% increase) during indirect stimulation with supramaximal shocks. When this is repeated with a muscle in advanced local tetanus and direct muscle stimulation, no increase in the twitch tension is observed. Similarly, no increase in the twitch tension is observed in a chronically denervated muscle. These results establish still another similarity between these two states.

The phenomenon of post tetanic potentiation has been a subject of considerable study, Gutman et al (1937), Feng et al (1937-41), Brown and Fuller (1938), Walker (1951), Feng and Li

(1941) using maximal single shocks, reported that eserine potentiation and post tetanic potentiation are essentially similar. They interpret both the responses as being due to a repetitive discharge of the motor nerve endings. They also state that the potentiation which follows the tetanic stimulus and eserine are essentially alike.

Feng and Li (1941) differentiate the motor nerve ending from the rest of the nerve fibres on the basis of the ability of motor nerve endings to respond repetitively with eserine. This seems to be in agreement with the work on prostigmine by Riker (1946). However, Walker (1951) suggests an alternative explanation for the post tetanic potentiation as being caused by an increase in the contractile strength of the component muscle fibres.

The results of the present investigation - i.e. the post tetanic facilitation and eserine potentiation, no matter what the mechanism may be in normal muscle, is abolished in advanced local tetanus and chronic denervation. This in itself is a significant finding. This substantiates the conclusion drawn so far, that in advanced local tetanus a progressive neuromuscular deterioration is taking place. The absence of post tetanic facilitation and eserine potentiation in both local tetanus and chronic denervation may be due to a disturbance or decrease in Ach and cholinesterase, Harvey (1939), Schaefer (1944), Werle and Stuttgen (1942), Ammon (1943), Cannon and Rosenblueth (1949), Strombled (1956), Beckett and Bourne (1957), Snell and McIntyre (1955).

Responses to Pharmacological AgentsAcetyl choline, Decamethonium and Succinylcholine

When these "depolarizing agents" are administered in small quantities (0.5 ug-1 ug) by distant arterial route in the muscle in progressive local tetanus (12-30 days), there occurs an immediate "shower" of fibrillatory potentials. This shower of electrical activity lasts for varying periods of time depending upon the dose of the agents and returns to preinjection level shortly. When a larger dose (50-100 ug) of these agents is used the shower of activity appears but suddenly comes to a standstill after about 25-30 seconds and an "electrical silence" is obtained. An exactly similar phenomenon is obtained when similar doses of these agents are injected into a muscle in chronic denervation (3-20 days). This type of sensitivity of the denervated skeletal muscle to chemical agents (especially transmitter and depolarizing agents) is a well-known phenomenon. In fact, Cannon in 1939 stated that whenever an organ was deprived of its nerve supply it became sensitized to chemical agents and, in particular, to the transmitter substance of the degenerating nerve. The change in chemical sensitivity following denervation is a generalized phenomenon occurring in various kinds of tissues such as skeletal muscle, smooth muscle, glands, etc. Cannon and Rosenblueth (1949), Emmelin (1959), (1961), Thesleff (1960) and others. Chronically denervated mammalian skeletal muscle is 100-100,000 times more excitable to Ach, Brown (1937), Tower (1939), Nicholls (1956) and

others. A close correlation between the onset and time course of Ach sensitization and fibrillation potentials after nerve sectioning, has been documented by Lucó and Eyzaguirre (1955).

The "depolarization" effect of excess of Ach, decamethonium, succinylcholine etc in the denervated muscle, as preported by Jarcho (1950), Zaimis (1951) and Riker (1957) was also observed in advanced local tetanus and chronic denervation experiments in the present investigation. This close correlation in the responses of these agents in local tetanus and denervation is strong evidence for the similar nature of the fibrillations obtained during the course of these two phenomena.

#### d-Tubocurarine

Subparalytic to paralytic doses (100 ug-250 ug) of d-Tubocurarine when administered by distant arterial route was ineffective in all experiments, on the spontaneous fibrillation potential activity of both local tetanus and denervation. However, in two experiments with very high doses (500-1000 ug) of d-Tubocurarine (under artifical respiration) a decrease in amplitude and frequency of the fibrillations were seen. These results confirm the reports of earlier investigators, Langley and Kato (1914-15) Solandt and Magladery (1940), Eccles (1941) and others, who had reported lack of change in fibrillation potentials to d-Tubocurarine in denervation. McIntyre (1945) however, working with denervated muscle of dogs, found that while blocking doses of d-tubocurarine did not affect the fibrillations very large doses

administered by close arterial injection produced a cessation of activity. Jarcho et al (1950, 1952) also reported a similar situation in rats. The results of the present investigation, with high doses, also show a similar trend. However, in none of the experiments reported here, was the typical electrical silence such as that obtained with Ach,  $C_{10}$ , and succinylcholine, observed with  $\delta$ -Tubocurarine.

### Cholinesterase inhibitors

#### Eserine, DFP, TEPP:

These agents in doses 50-200  $\mu$ g/kg did not exert any significant effect on the spontaneous fibrillations of local tetanus or chronic denervation. However, immediately after their injection, the innervated and non-treated muscles showed wide spread twitchings. No such twitching was seen in denervated or tetanus treated muscle. With DFP and TEPP occasional bursts of fibrillation potentials were observed superimposed on the routine background fibrillation activity.

Administration of 0.5-1.0  $\mu$ g of Ach after the treatment with these cholinesterase inhibitors showed a slight increase in the duration of the "shower" of activity. But the increase did not appear to be significant. When larger doses (5-10  $\mu$ g) of Ach were injected the potentiation of the effect was prominent.

Thus electrophysiologically and pharmacologically the muscle in advanced local tetanus exhibits a close similarity to that in chronic denervation. Lucco and Byzaguirre (1955) have

shown in the denervated mammalian skeletal muscle a close correlation between the development of hypersensitivity to Ach. and the presence of spontaneous fibrillation potentials. Riker et al (1957) used this phenomenon of fibrillation potentials as a test procedure for evaluating the depolarizing agents based on their property to induce, on intraarterial injection, an increased showering in the spontaneous fibrillation potentials in the denervated muscle. The results of the present investigation points out a close parallelism between the effect of Ach,  $C_{10}$ , and succinylcholine on the muscle in advanced local tetanus on one hand and that on the muscle in chronic denervation on the other. This suggests the development of hypersensitivity in the former.

Considerable work has been reported during the last 30 years on the phenomenon of fibrillation and hypersensitivity in chronic denervation due to neural degeneration and in so-called "pharmacological denervation", Brown, (1937) Cannon and Rosenblueth (1949), Rosenblueth and Luco (1937), Solandt and Magladery (1942), Kuffler (1943), Nicholls (1956), Emmelin (1952, 1961), Axelson and Thesleff (1959), Miledi (1959), Li et al (1959), Elmquist and Thesleff (1960) and others.

The suggested mechanisms underlying this phenomenon of fibrillation are: (1) a decreased rate of enzymic hydrolysis (2) changes in the excitability and the electrical properties of the muscle membrane (3) changes in the number and affinity of the receptors at the end plate (4) spreading of the receptor surface

throughout the entire muscle membrane.

The first suggestion that the decrease in cholinesterase activity in the denervated muscle might be the cause of supersensitivity cannot in itself account for this phenomenon, since, other agents, not known to be involved in cholinesterase activity like, Decamethonium, Succinylcholine etc. are also highly active. Furthermore, no correlation is observed between the total cholinesterase and degree of supersensitivity, Brooks and Chipman (1952), Brooks and Myers (1952).

Reduction in membrane permeability with the resultant increase in the membrane resistance, thereby requiring less potential change for depolarization (Nicholls, 1956) can at the most only partially account for the observed hypersensitivity. Further, this property would not explain the hypersensitivity to injected Ach.

Kuffler's (1943) suggestion that the sensitivity of Ach receptors at the end plate increases after denervation has been questioned by Thesleff, who observed no such increased sensitivity at the end plate region following a strictly localized (iontophoretic) application of Ach in that region. Axelson and Thesleff (1959), Miledi (1959) believe that even after denervation the end plate receptors maintain their original responsiveness to iontophoretically applied drugs and the only observable change is the increase in the responsiveness of the muscle cell surface to Ach. Such an increase of the region, sensitive to Ach, was also postu-



lated by Perry and Zaimis (1954). This view is also compatible with the observation that fibrillation potentials, shortly after the beginning of denervation, are confined to the end plate region. They then spread towards the tendons of the muscle fibres as the Ach sensitive area increases with the progress of denervation, Luco and Eyzaguirre (1955).

Several reports are available in the literature (Emmelin, 1961) which indicate that various drugs, interfering with the storage, release and action of chemical transmitters, may increase the reactivity of the effector cells if they are allowed to act for a long time. It is interesting to note here, that there is a great resemblance between the supersensitivity caused by denervation and that caused by the prolonged treatment with these transmitter-interfering drugs. It seems justified to extend the law of denervation and assume it to be valid not only for surgical denervation but for pharmacological "denervation" as well. For instance, agents like atropine, botulinum toxin, reserpine etc. have been shown to produce so-called pharmacological "denervation" and hypersensitivity.

Thesleff (1960) has shown that botulinum toxin produces a supersensitivity of mammalian skeletal muscle, which closely resembles that caused by the sectioning of motor nerve. In botulinum intoxicated fibres, just as in denervation fibres, the area sensitive to Ach was found to be increased far beyond the confines of end plate, and to extend to the whole muscle cell, Axelson and

Thesleff (1959). The time course for the increase in Ach sensitive area was the same for both denervated and botulinum toxin treated muscle. It has also been shown that botulinum toxin neither affects the ultra structure of motor nerve endings nor the transmitter formation and storage, Thesleff (1960). Further, in these two states, nerve stimulation caused no end plate potentials and also the spontaneous miniature potentials were absent. Presumably then, botulinum toxin selectively blocks the mechanism responsible for the release of chemical transmitter agent from the nerves, Procks, (1956) Lammanna (1959), and Stevenson (1958). It is therefore, postulated that lack of transmitter agent, and not nerve degeneration per se, is responsible for initiating the process which causes the high and uniform chemical sensitivity of these muscles, Thesleff (1960), Emmelin (1961). The possibility that the supersensitivity may result from lack of transmitter had, in fact, been considered long ago. In 1934 Dale had pointed out that the depot of transmitter at the nerve endings disappears when the nerve degenerates and wrote: "We may note in passing, the probability that the exaggerated sensitiveness of the denervated effector cells, to the artificial application of chemical transmitter, may be conditioned by this disappearance of its depot and the failure of its normal release."

Questions have been raised - (1) is the hyperactivity of denervation an inherent property of skeletal muscle itself, having existed from intrauterine life? (2) is denervation a restoration

or return to an inherent function of muscle fibre, which exists in its infantile preinnervation state? Studies on embryonic muscle by Marinacci (1959), tissue culture explants by Li et al (1959), surgical and pharmacological "denervation" by Thesleff (1960), Emmelin (1961) etc., seem to indicate that absence of nerve connection, and/or lack of transmitter release, converts the whole muscle membrane into an Ach-sensitive surface. These observations would suggest that a uniform chemical sensitivity of the muscle membrane is its original state and that upon anatomical innervation this preinnervation state is modified by the influence of nerves.

Electrophysiological and pharmacological data from advanced local tetanus and chronic denervation have established a similarity between these two states as far as responses to electrical stimuli, fibrillation potentials, and responses to chemical agents are concerned. Perhaps, the basic mechanism underlying these two processes viz. degeneration, denervation and advanced local tetanus, may be identical, i.e. the interference with the formation and/or release of the transmitter agent from the motor nerve ending. The toxin may bring about this change by interfering with the enzymatic or metabolic process. This statement receives some support when it is realized that the quantity of the toxin necessary to produce this effect, is extremely minute and the time required to bring about this change is comparatively long. This long latency suggests that the toxin may perhaps

interfere with the synthesis of essential enzyme systems so that the typical symptoms would not set in until these essentially remote systems, have become depleted. Indications for the interference of Ach and cholinesterase metabolism at the nerve ending and myoneural junction were given by Harvey (1939). Ambachi et al (1948) observed that following the injection of tetanus toxin into the anterior chamber of rabbit eye, the pupil became widely dilated, light reflex was lost, and the sphinctor pupillae ceased to respond to stimulation of the third cranial nerve. Similar observation was made with botulinum toxin by Ambachi and his co-worker (1949). Schaefer (1944) also described a slight reduction in the cholinesterase activity in the muscle treated with tetanus toxin. It appears therefore, reasonable to hypothesize that the fibrillation potentials and the changes in the responses to chemical agents, in advanced local tetanus could be the result of a metabolic or functional lesion at the myoneural junction.

The action potentials obtained from the sciatic nerve of the rabbit's hind limb from muscles in advanced local tetanus showed that such nerve transmits trains of action potentials which are not significantly different from the nontreated ones. Acheson and his co-workers (1942) also reported no changes in the action potentials of the nerves supplying muscles in advanced local tetanus. Further, the fact that the muscle in local tetanus completely recovers within about 8-10 weeks after the onset of local tetanus is concrete evidence that degeneration of the anterior

horn cell is not involved in any degenerative process. It seems therefore, that the probable site of lesion in this case is the myoneural junction. Further work will be necessary to elucidate the exact details of the changes which accompany this phenomenon.

## SUMMARY

Local tetanus was produced in the tibialis anticus muscle of the rabbit by intramuscular injection of a minute quantity of tetanus toxin. Electrophysiological and pharmacological studies were made on the affected muscle at various intervals of time following the injection of the toxin, and compared with those of denervated muscle.

The following were the salient features:

Electromyography

- 1) The muscle in early local tetanus (1-5 days) exhibited a state of unremitting stiffness and hyperreflexia accompanied by a high degree of electrical activity.
- 2) The disappearance of this enhanced electrical activity in the muscle in early local tetanus after treatment with central depressant agents or nerve sectioning demonstrates its dependence on the spinal cord.
- 3) As the local tetanus progressed (5-8) the EMG features changed. Polyphasic potentials, positive sharp waves, and occasional fibrillation potentials were the major findings, instead of large number of normal motor unit potentials. These are the established features of a neuromuscular lesion.
- 4) With further progress of local tetanus (10-35 days) the electromyogram consisted entirely of fibrillation potentials which

were identical in every respect to those of denervated muscle.

5) As further time progressed the EMG features showed a reverse sequence i.e. the spontaneous fibrillation potentials were replaced by positive sharp waves, polyphasic potentials and occasional motor unit potentials. These signs are analogous to those obtainable during regeneration following neural damage. Similar features were obtained from denervated muscle upon regeneration of the nerve.

### Electrodiagnosis

1) As the duration of local tetanus increased (5-30 days), strength-duration curves of the muscle showed an increased threshold, especially with lower stimulus duration, in a manner very similar to the curves in chronic denervation. The strength-duration curves in local tetanus were complex containing two segments with discontinuities or kinks appearing around 6-12 days and again around 45-55 days after the injection of the toxin. These findings indicated an incomplete or partial denervation and/or partial reinnervation respectively. Such finds are characteristic of partially denervated or reinnervated muscle.

2) With the progress of local tetanus and denervation, Rheobase showed an initial rise and a subsequent fall. A return to normal, with recovery, was also found in both cases.

3) Chronaxie showed an increase of 70-90 times the normal, with the progress of both local tetanus and chronic denervation and a return towards normal as the recovery ensued.

- 4) Changes in galvanic-tetanus ratio and responses to repetitive stimulation were found to be comparable both in local tetanus and chronic denervation.
- 5) Isometric contractions of the muscle to nerve stimulation gradually decreased and were completely abolished as the tetanus progressed (6-12 days).
- 6) Post tetanic potentiation of the muscle was absent in both local tetanus and chronic denervation.
- 7) Responses of the muscle to direct stimulation before and after a tetanic stimulus were identical both in local tetanus and chronic denervation.

#### Responses to Pharmacological Agents

The studies on the effect of pharmacological agents on the spontaneous fibrillation activity in advanced local tetanus and chronic denervation revealed the following:

- 1) Ach, Decamethonium, and Succinylcholine in minute doses (0.5-1 ug) brought about an immediate increase of spontaneous fibrillatory activity of the muscle. With higher doses, the increase was followed by a complete cessation of activity which was eventually, gradually restored to preinjection level.
- 2) Cholinesterase inhibitors like physostigmine, DFP, TEPP did not exert any effect on the spontaneous fibrillation potentials of local tetanus or chronic denervation. However, the actions of Ach were prolonged by these agents.
- 3) d-Tubocurarine in paralytic doses had no effect on the spon-



taneous fibrillations of local tetanus and chronic denervation.

On the basis of this close similarity of electrophysiological and pharmacological data between the muscle in local tetanus and chronic denervation it was concluded that progressive local tetanus brings about, in the affected muscle, a situation similar to chronic denervation. Further, the spontaneous fibrillatory electrical activity in both the cases are indistinguishable from each other.

The exact mechanism by which this "denervation" is brought about, is not indicated by this investigation. However, the results of the present investigation are discussed in the light of available data obtained with other agents, for instance, botulinum toxin, which is known to cause similar "denervation". Presumably the "denervation" caused by tetanus toxin is through an interference with the synthesis or release of the transmitter agents and/or an interference in the related enzyme systems at the myoneural junction.

The controversy over the central versus the peripheral action of tetanus toxin is reviewed and evaluated in the light of the new information made available from this study. Effects of tetanus toxin at both sites seem tenable in view of the total knowledge of its action now available.

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