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PHARMACOLOGICAL AND ELECTROPHYSIOLOGICAL EFFECTS
OF PROLONGED NEUROMUSCULAR IMPULSE DEPRIVATION IN SKELETAL MUSCLE

by

Emery Dean Robert

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

June 1970

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BIOGRAPHY

Emery D. Robert was born in Dresden (Germany) and immigrated to the United States in 1949; he is now a U.S. citizen. He received his Real-Gymnasium Baccalaureate (in Debrecen, Hungary) and then attended Tisza Istvan University (Debrecen, Hungary) where he studied medicine, chemistry and law; here, he was awarded the Degree of Doctor of Laws and Jurisprudence, in 1945. He continued the study of chemistry at the Bolyai University of Sciences (Cluj, now Roumania) and at the Pazmany Peter University (Budapest, Hungary), and completed his studies for the Degree of Doctor of Science (Biochemistry) at Bolyai University, in 1946.

After coming to the U.S., he was engaged in research and development in the cosmetics and chemical specialties fields, and, as a biochemist and scientific administrator, he reached the position of Director of Research (Lady Esther Co., Chicago).

In the Fall of 1960, he began his graduate studies in the Department of Pharmacology of the Stritch School of Medicine. He had been a graduate assistant in this Department until his leave of absence in order to study medicine. He received his M.D. Degree from the University of Illinois, in 1967; completed his internship at Michael Reese Hospital and entered Residency Training in Neurology at Northwestern University. His specialty training was interrupted in order to complete research and compilation of data in connection with a NIH grant, and to write his doctoral dissertation, both at Loyola University Stritch School of Medicine, under the aegis of Doctor Y. T. Oester.
He is the Co-Principal Investigator for NIH Grant NB-05728, "Denervation and Reversible Nerve Block", with Dr. Oester as the Principal Investigator.

He is co-author of the following publications:


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CHAPTER I
REVIEW OF THE LITERATURE

THE NEUROTROPHIC CONCEPT: AN INTRODUCTION

The characteristic electrophysiological and pharmacological changes which occur in skeletal muscle after surgical nerve section have been well documented. Cannon (1939), in his classical work, formulated the "Law of Denervation" in which he stated that when, in the chain of efferent neurons, the continuity is disrupted, an "...increased irritability to chemical agents develops in the isolated structures...". Cannon and Rosenblueth (1949) further elaborated on this concept, extending it to be valid for all innervated structures, such as skeletal muscle, smooth muscle, glands and ganglia; the familiar term, "denervation supersensitivity", was first used by these investigators. Cannon and Rosenblueth (1949) suggested that "denervation supersensitivity" would develop in the aforementioned organs or structures whenever their efferent chain of neurons suffers discontinuity; they postulated that the denervation changes may be induced because of (a) the prolonged absence of activity in the denervated tissue(s); (b) the lack of release of the chemical transmitter; or (c) the absence of a "trophic" factor which normally exerts its beneficial effects through the intact nerve and thus maintains the integrity of the innervated structure.

Earlier, Claude Bernard (1880) voiced this concept by noting that "the excitability of all tissues seems to augment when they are separated from nervous influences which dominate them". This very broad concept
has been axiomatic for many decades; whether it implies the "law of
denervation" or perhaps something else or something broader, invites some
reflections. The complex structural and functional arrangement of the
nervous system, particularly, but not exclusively, the central nervous
system, is operational through a formidably precise organization. The
multitude of excitatory and inhibitory pathways and their complex inter-
actions are overwhelming. According to the classical concept of Hughlings
Jackson (1884), a destructive lesion at a higher level of the central
nervous system will not only result in the loss of control of the higher
over the lower level(s) but also will bring about a "release" (or a
"disinhibition") of the latter, with a resultant increase in activity.
The "law of denervation", as it was originally formulated by Cannon (1939),
has been generally accepted in its application to peripheral structures.
In the recent past, however, "central denervation supersensitivity" also
has been reported, for example by Harvey and Lints (1965), who observed
this phenomenon in the medial forebrain bundle of rats, following surgical
lesions in this region.

In attempting to elucidate some aspects of the "neurotrophic" influence
of the nerve supply on the cells of the effector organ, it seems important
to distinguish between "denervation supersensitivity", which develops
because of some impairment of this "trophic influence", and the heightened
activity in a "decentralized" organ, which occurs as a consequence of
"release". The latter phenomenon is due to the cessation of inhibitory
mechanism(s) which keep the organ or structure under a control of balance.
This differentiation, however, is rather difficult, particularly within the regions of the central nervous system. Peripherally, where the structures may be more precisely isolated and thus observed, such distinction may be possible.

In the autonomic nervous system, for example, many studies have dealt with the effect of interference with the neuro-effector pathways, or with neurotransmission, per se. One of the earliest observations reported in this area was that by Henderson (1923) who used atropine as the blocking agent in order to prevent the physiological actions of the neurotransmitter on the submaxillary gland of the cat. Nickerson and House (1958) compared the sequelae of surgical denervation of the nictitating membrane of the cat with the effects of a prolonged blockade, by Dibenzyline, of this membrane. These authors concluded that the absence of the physiological neuromyial mediator, secondary to such different procedures, caused denervation sensitization of comparable degrees in both preparations. Nickerson (1962), in a personal communication, re-emphasized his view, stating that "...functional denervation by pharmacological blockade...seems consistently to have much the same effect as anatomical denervation."

There are numerous reports in the literature dealing with surgical or pharmacological interference with the physiological functions of various "nerve-effector organ" units in the autonomic nervous system. In a review of these reports, Emmelin (1961) indicated that various drugs (if allowed to act for a prolonged period of time), by interfering with the storage, release or action of the chemical transmitter, will cause an increased
sensitivity of the effector cells to the neurotransmitter. Emmelin et al. (1951) examined the effects of prolonged administration of atropine and pilocarpine on the submaxillary gland of the cat, and found that the structural changes and the increased adrenaline sensitivity were similar to or identical with these changes following surgical transection of the chorda tympani. Emmelin and Stromblad (1957) experimented with nerve impulse deprivation of glands and smooth muscle; they concluded that a pharmacological blockade of the innervated organ will cause alterations analogous to those which follow surgical nerve section. According to this view, "pharmacological decentralization" (i.e. a preganglionic blockade) or "pharmacological denervation" (i.e. a postganglionic blockade) will result in chemical supersensitivity of a degree equivalent to that caused by surgical interruptions of the neural pathway in the corresponding region.

Emmelin (1952) prevented the nicotinic effects of acetylcholine and induced "paralytic secretion" in the submaxillary gland of cats. This form of denervation supersensitivity was attributed by the author to the "isolation" of the effector cells from central nervous influence. Again, Emmelin (1959), re-emphasized the validity of Cannon's Law of Denervation (1939) by stating that this concept is applicable when the "denervation" is brought about by pharmacological means. Yet, earlier, Emmelin and Muren (1950) reported that "...their experiments do not support the theory that the normally innervated gland is subjected to a 'trophic' influence... nor can the sensitization (of the denervated gland) occur consequent on a suppression of the liberation of the transmitter substance." In fact,
Konzett and Rothlin (1953) reported absence of supersensitivity after prolonged treatment of the nictitating membrane of the cat with hexamethonium; an experiment which was employed by Emmelin in his 1959 work. Perhaps, further examination of the neurotrophic concept is indicated. In a recent report, Brown (1969) actually challenged the concept of denervation supersensitivity in his report by concluding that the denervated superior cervical ganglion of the cat "...does not fully obey Cannon's (1939) 'law of denervation'."

In the skeletal muscle, supersensitivity is particularly marked and invariably follows surgical denervation. The denervation changes are well documented (Brown, 1937; Tower, 1939b; Desmedt, 1949). It seems certain that anatomical continuity of the motor nerve and contiguity of the neuromyal apparatus are sine-qua-nons for the functional integrity of skeletal muscle. Although the nature and the mechanism of this "trophic" neural influence is not known, many reports and excellent reviews and monographs have extensively treated the various aspects of denervation and the influence of motor innervation on the skeletal muscle; for example, Tower (1937 and 1939a); Eccles (1941a, 1944, 1963); Desmedt (1959); Gutmann (1959, 1964); Gutmann (ed., 1962); Gutmann and Hník (eds., 1963); Thesleff (1960b); Sharpless (1964); and others. These references cited do not provide an all-inclusive review; such thorough review of this topic would be formidable in scope. In the present study, only a short review of the pertinent background will be given. It is hoped that serious omissions have been avoided and that some of the references cited will not imply
that similar or related reports are less significant. In general, it is hoped that the purpose of this review has been served by giving a comprehensive and comprehensible description of events and concepts which motivated this research.

**NEUROMUSCULAR TRANSMISSION**

The physiology of neuromuscular transmission and various pharmacological agents affecting the skeletal neuromyal junction have been extensively studied in the preceding decades. Many functional aspects and the morphology of this synapse have been adequately explained and are universally accepted. There are, however, still many facets of the neuromuscular transmission mechanism which are still unknown and several which are controversial. Excellent recent reviews deal in some or in extensive details with the physiology and pharmacology of junctional transmission (Thesleff and Quastel, 1965; Nastuk, 1966; Karczmar, 1967). It seems universally accepted that the mechanism of transmission at the vertebrate neuromyal junction is cholinergic in nature (Eccles, 1964; Karczmar, 1967). The major events of transmission, which are presynaptic, synaptic, and postsynaptic, and which lead to the physiological activation of the vertebrate skeletal muscle, may be grossly summarized as follows. When an appropriate and adequate stimulus (volitional or experimental, such as an electrical stimulus) is applied to the motor nerve, it initiates a propagated nerve action potential leading to the depolarization of the motor nerve terminals. This, in turn, initiates release of ACh from its storage in the synaptic vesicles. ACh diffuses
across the synaptic cleft, combines with receptor sites in the postsynaptic membrane and initiates membrane depolarization. This excitation, i.e. the propagated muscle potential, brings about a complex series of events within the contractile elements of the muscle which is ultimately manifested in muscle tension.

A diagrammatic scheme of the synthesis, storage, and release of ACh (in the superior cervical ganglion of the cat) was presented by Birks and MacIntosh (1961). Several investigators dealt with the possible mechanism which renders ACh release adequate for muscle activation, under normal and pathological conditions (Desmedt, 1966; Nastuk, 1966; Thesleff, 1966). The physiology of the neuromuscular junction was extensively treated by Eccles (1964); and the dependence of ACh release on the presence of cations (Mg, Ca, Na) has been described by several investigators (Katz, 1962; Katz and Miledi, 1965 and 1967b; Gage and Quastel, 1965; Thesleff and Quastel, 1965). Details of these aspects of neuromuscular transmission will not be reviewed in this report. An important phenomenon at the neuromuscular junction, however, should be reviewed because of its possible role in the mechanism of the trophic neural influence on skeletal muscle. At the neuromuscular synapses of vertebrates, a spontaneous electrical activity was described by Fatt and Katz (1952). These small depolarizations of the postsynaptic membrane, occurring in a random temporal sequence in the resting muscle, give rise to miniature end-plate potentials (m.e.p.p.'s) which are about one-hundredth of the synaptic potential (end-plate potential) produced by a presynaptic impulse.
Evidence indicates that these m.e.p.p.'s are produced by the spontaneous release of multimolecular ("quantal") units of ACh (Del Castillo and Katz, 1955; Katz, 1958; Katz, 1962); each synaptic vesicle liberating one quantal unit. When the muscle is not at rest, i.e. upon the arrival of a propagated nerve action potential at the presynaptic terminals, the synchronized release of several hundred synaptic vesicles liberates sufficient ACh to produce the end-plate potential (De Robertis, 1964).

It has been proposed (Johns and Thesleff, 1961) that prolonged inactivation of the skeletal muscle, with an intact motor nerve, is protected from the development of denervation supersensitivity by the presence of m.e.p.p.'s.

**DENERVATION SUPERSENSITIVITY**

In the chronically denervated muscle, the supersensitivity changes are especially pronounced and the characteristic alterations which follow denervation have been well documented. The inevitable consequences of surgical (chronic) nerve section become manifest by a triad of classical signs: (1) the occurrence of spontaneous fibrillation potentials in the muscle (Denny-Brown and Pennybacker, 1938; Tower, 1939b); (2) alterations in the electrical excitability constants of the muscle (Desmedt, 1949); and (3) an increased sensitivity of the muscle to applied pharmacological agents, especially to its neurotransmitter (Brown, 1937). In addition, atrophy of the muscle invariably follows chronic denervation; the muscle will diminish in volume and several histopathological changes can be demonstrated in the muscle and within its fibers (Tower, 1935 and 1939a;
Sunderland and Ray, 1950; Adams et al., 1954; Gutmann and Zelena, 1962; Hnik, 1962). The metabolic reactions to denervation, in the muscle, are numerous, and the biochemical changes which accompany denervation have been impressively documented (Gutmann, 1959; Bass, 1962; Zak, 1962; Gutmann, 1962). The electrophysiological and pharmacological changes which are inevitable after surgical section of the motor nerve, have been reported to occur after a variety of procedures affecting the motor nerve and/or the skeletal muscle in the absence of actual motor nerve section (Thesleff, 1960a; Fudema et al., 1960a and 1960b; Prabhu and Oester, 1962 and 1963; Prabhu et al., 1962).

Consequences of Surgical Denervation

Electromyographic Changes: Fibrillation

Electromyography (EMG) is a technique for recording the intrinsic electrical activity within the skeletal muscle. Its clinical application for the diagnosis of denervation and other muscle abnormalities was emphasized by Weddell et al. (1944) and the instrumentation and examination methods are described by Rodriguez and Oester (1956). In the normal skeletal muscle, neuromuscular function is represented by the production of motor unit potentials on the EMG oscilloscope. Although the parameters of normal motor units vary in different skeletal muscles, the predominant individual action-potentials are di- and triphasic; the voltage ranging from 100 to 2000 microvolts and lasting for a period of 2 to 10 milliseconds. During vigorous contractions of the muscle, numerous motor units discharge at random and the summating effect prevents measurements of a
single unit discharge. At rest, the normal skeletal muscle reveals an iso-electric baseline on the EMG oscilloscope, which is generally referred to as "electrical silence". When the muscle is denervated, and in various diseases, for example in myopathies, in anterior horn cell disease, in tetany, and others, abnormal motor unit potentials (i.e. changes in amplitude, frequency or duration) are seen on the oscilloscope.

Within a few days after surgical section of the motor nerve, fine spontaneous movements of the muscle fibers appear. Schiff (1851) first observed these fine movements and termed them "fibrillation". The electrical silence which appears immediately after nerve section, is replaced by "irritation" potentials (within a day or so) and on the third or fourth day, following denervation, spontaneous and persistent "fibrillation potentials" appear. These potentials have an amplitude of approximately 50-300 microvolts and a duration of 0.5-2.0 milliseconds; they are monophasic or biphasic, have a frequency of 2-3 per second but usually closer to 10 per second. They are characterized by a short high tone, like a sharp, high-pitched click. That fibrillation develops after motor nerve section, at the time when degenerative changes occur in the muscle fibers, was reported by Langley and Kato (1915a). Fibrillation appears after lesions of the peripheral motor neuron, but it does not occur in muscle atrophies which follow tenotomy or immobilization (Tower et al., 1941; Solandt and Magladery, 1940; Johns and Thesleff, 1961).
The occurrence of spontaneous fibrillation potentials in a denervated skeletal muscle has not been adequately explained. Several theories in the past have pointed to the possible role of the neurotransmitter and/or nerve impulse activity as being essential for physiological maintenance of the muscle. Since fibrillation generally occurs together with pharmacological supersensitivity, it was proposed that fibrillation is due to the markedly lowered threshold of the denervated muscle to traces of ACh in the blood or in surrounding tissues (Denny-Brown and Pennybacker, 1938; Bergamini et al., 1955). The influence of the spontaneous fibrillation by close-arterially injected ACh was first pointed out by Frank et al. (1922). Brown (1937) reported a marked "shower-like" increase of the characteristic potentials following ACh injection. Fibrillation activity appears after a certain time following the nerve section. This time is variable, dependent on the species of animal and on the type of the muscle (Langley and Kato, 1915a and 1915b; Feinstein et al., 1945; Desmedt, 1950). Under identical conditions, in the cat, the spontaneous fibrillary activity appeared earlier when the peripheral nerve stump, innervating the muscle, was sectioned nearer to the muscle (Luco and Eyzaguirre, 1955). In fact, both the onset of fibrillation and of increased sensitivity to ACh, paralleled the time course of the progressive neuromuscular failure. Concomitantly, the miniature end-plate potentials also disappeared (Luco and Eyzaguirre, 1955). These changes point to some association between the occurrence of fibrillation and the cessation of the nerve influence. Denny-Brown and Brenner (1944), for
example, suggested that fibrillation will not occur, even in the prolonged absence of neuromuscular impulses, provided that the anatomical and histological integrity of the neuromuscular pathway is preserved.

Earlier, a similar conclusion was reached by Tower et al. (1941), who investigated the cause of fibrillation by comparing surgically denervated and "functionally denervated" preparations. They achieved inactivation of the muscle by isolation of the spinal cord from afferent impulses, leaving the motor innervation intact. Fibrillation occurred when the motor nerve was sectioned but did not occur in the relatively inactive muscle (with intact motor nerve). These investigators concluded that the neurotrophic influence is exerted through the intact motor nerve. The effect of a prolonged and reversible simple nerve impulse deprivation (without any destructive procedure) on the electromyographic potentials of the skeletal muscle was first reported, in a preliminary study, by Robert and Oester (1964); this study formed the basis for the research described in this dissertation.

Changes in Electrical Excitability of the Muscle

Electrodiagnostic (EDX) changes (i.e. alterations in the electrical excitability constants of the skeletal muscle), which follow surgical denervation, have been equally well documented (Watts, 1924; Pollock et al., 1945; Desmedt, 1949; Nicholls, 1956). Electrodiagnosis employs several electrophysiological procedures for determining excitability changes in muscle. The electrical constants are usually determined by measuring the rheobase and chronaxie, plotting strength-duration (S-D) curves, assessing
responses of the muscle to repetitive (electrical) stimulation, and some other procedures. (For definitions and descriptions of rheobase, chronaxie, S-D curve and repetitive stimulation, see Methods.)

Duchenne de Boulogne (1855) was first to observe that responses of a denervated muscle to electrical stimulation are different from those of a normal muscle. Lapicque (1909) developed the concept of rheobase as a measurable threshold of excitation; he also defined and employed the word "chronaxie" to denote the minimal duration of current required to stimulate, at twice the rheobase intensity. Chronaximetry, clinically instituted by Lapicque (1921) and Bourguignon (1923), has become a widely used electrodiagnostic method for the examination of muscle atrophies, although its value, as an exact index of tissue excitability, is questioned by some investigators (Rushton, 1930; Grundfest et al., 1956). Grundfest et al. (1956) maintained the view that a normal chronaxie, corresponding to a small number of innervated muscle fibers, may be found in a partially denervated muscle, even though a great number of the muscle fibers are denervated. Woodhall et al. (1956) suggested chronaximetry as a suitable method for determining a denervation state but not the early re-innervation state; the return of voluntary movements in a previously denervated muscle precedes restitution of normal chronaxie values. Normal rheobase is generally between 0.8-1.0 milliampere; it becomes elevated to 1.2-1.3 milliampere shortly after denervation and then progressively falls to a low level of 0.2 or less, by about the tenth day after denervation. Normal chronaxie values are generally below 1.0 millisecond but chronaxie
becomes markedly elevated after denervation, usually above 10 milliseconds and up to 100 milliseconds.

The strength-duration curve is probably the most reliable electrodiagnostic examination based on excitability measurements. Adrian (1916) found that S-D curves from a normal muscle give an asymptotic hyperbole, and that the normal curve is continuous. In contrast, the curve from a denervated muscle is moved to the right and upward, and during reinnervation the curve becomes "indented" or "discontinuous". In a series of temporally separated S-D curves, the point of indentation is of clinical diagnostic significance: its appearance generally heralds the return of voluntary movements by 4 to 6 weeks (Ritchie, 1954).

Electrodiagnosis with repetitive stimulation was found to give reliable information about denervation (Pollock et al., 1947). In the normal muscle, repetitive stimulation, at all commonly used frequencies (500, 166, and 91 cycles per second), require about the same current to produce tetanic contraction. In the denervated muscle, however, progressively greater amounts of current are required as the frequency of stimulus decreases. In addition, the current required, at all three frequencies, increases pari passu with the denervation course.

Pharmacological Changes

The chemical supersensitivity of the denervated skeletal muscle is particularly marked. One of the earliest reports, regarding increased sensitivity to ACh in the denervated muscle, was that by Dale and Gasser (1926) showing that ACh produced a slow contraction, i.e. a contracture in
the denervated but not in the innervated muscle. Studies in regard to quantitated chemical supersensitivity, that is a change in threshold, were first reported by Brown (1937) on the gastrocnemius muscle of the cat, following section of the sciatic nerve. Subsequently, Rosenblueth and Luco (1937) showed that in the denervated muscle close-arterial injection of minute amounts of ACh results in a dual mechanical response: a quick contraction, followed by a slow contracture. Brown (1937) and several other investigators (Rosenblueth and Luco, 1937; Reid and Vaughan-Williams, 1949; Nicholls, 1956) showed that denervated mammalian skeletal muscle becomes 100 to 100,000 times more sensitive to close-arterially injected ACh than the muscle was prior to denervation. The heightened responsiveness of the denervated muscle is not limited to the transmitter; pharmacological agents such as nicotine, choline, and several agents which may have an effect on the cholinergic receptors, elicit a marked response in very small doses (Cannon and Rosenblueth, 1949). D-tubocurarine, for example, which causes no mechanical contraction in the innervated muscle, depolarizes the muscle after denervation (Bowman and Raper, 1964).

In the normally innervated muscle, ACh sensitivity is limited to the end-plate region. Following denervation, this restricted chemosensitive area becomes enlarged and the entire muscle surface becomes equally sensitive to ACh (Axelsson and Thesleff, 1959), the end-plate region, per se, retaining the degree of its original ACh-sensitivity (Elmquist and Thesleff, 1960). It is thought that the extension of chemical sensitivity is due to the spread of receptors over the muscle surface (Miledi, 1962).
In the normally innervated muscle fibers, the "junctional" ACh-receptors are limited to the end-plate region under the terminal arborization of the motor nerve; the presence of innervation, but not necessarily the presence of nerve impulses, is thought to prevent the extension of the receptor surface which occurs after denervation (Miledi, 1960a, 1962, and 1963).

Consequences of "Pharmacological Denervation"

In addition to surgical interruption of the motor nerve, several other procedures, in which the motor nerves were not severed, are known to bring about the characteristic changes of denervation supersensitivity. As mentioned before, the suggestion was made (Nickerson and House, 1958; Emmelin, 1961; Nickerson, 1962) that interference with the action of the neurotransmitter will bring about such changes; the phenomenon was termed "pharmacological denervation" (Emmelin, 1961).

Certain neurotoxins which alter the functional integrity of the neuromuscular mechanism or which affect the contiguity of the neuromyal junction, have been reported to induce denervation supersensitivity. Thesleff (1960a) reported that mammalian skeletal muscle, after treatment with botulinum toxin, developed supersensitivity to ACh. The degree of supersensitivity was equal to that observed after surgical denervation. Since no degenerative changes were observed at the neuromyal junction or in the nerve endings of the botulinum treated muscle, Thesleff attributed the change to prevention by the toxin of ACh release by the nerve endings.
Brooks (1954) and Brooks et al. (1955) reported that botulinum toxin inhibits the release of ACh from motor nerve terminals. Stromblad (1960) suggested that botulinum toxin interferes with cholinesterase activity in the muscle. It was proposed by Thesleff (1960a, 1960b) that the absence of m.e.p.p.'s, through the inhibitory mode of action of botulinum toxin, resulted in the removal of the trophic influence which normally would prevent extension of the ACh sensitive area over the entire muscle surface. This uniform chemosensitivity of the entire sarcolemma was reported by Axelsson and Thesleff (1959) to occur after surgical denervation and was attributed to the disappearance of m.e.p.p.'s in the denervated neuromyajunction. To support Thesleff's theory, Johns and Thesleff (1961) immobilized skeletal muscle by lower lumbar cord section and dorsal rhizotomy, leaving the motor nerve intact. The authors reported that there was "relatively" no change in the ACh-sensitive area and in the frequency of m.e.p.p.'s at the neuromyajunction of the immobilized muscle. They attributed the absence of supersensitivity to the presence of m.e.p.p.'s.

Tetanus toxin, acting peripherally as a "local tetanus", was reported to cause supersensitivity in the anterior tibialis muscle of the rabbit, following intramuscular injections of minute quantities (Prabhu et al., 1962a and 1962b). The mode of action of the toxin may be interference with ACh action at the neuromyajunction and/or with cholinesterase activity in the muscle (Harvey, 1939a). A more recent theory suggested inhibition of transmitter release (Brooks et al., 1955), similar to the
proposed mode of action of botulinum toxin (Brooks, 1954). Prabhu and Oester (1962) documented that the spontaneous fibrillation, which appeared as result of tetanus toxin treatment of the muscle, was identical in every respect to EMG changes seen after chronic denervation. Electrodiagnostic changes analogous to those following surgical denervation were also seen in the tetanus toxin treated anterior tibialis of the rabbit (Prabhu and Oester, 1963). The pharmacological supersensitivity of the muscle in local tetanus was also remarkably similar to that seen in chronic denervation, as reported by Prabhu, Oester and Karczmar (1962b). These investigators concluded that tetanus toxin, through its peripheral action, produced the phenomenon of "pharmacological denervation", resulting in the spread of the restricted ACh-sensitivity over the entire muscle membrane, in a manner identical to that seen in surgical denervation.

Experimental vitamin-E deficiency was reported to cause electromyographic (Fudema et al., 1960a) and electrodiagnostic (Fudema et al., 1960b) changes in the anterior tibialis muscle of the rabbit. The findings also included a separation of the motor end-plate complex from the muscle fibers, the latter still retaining its capacity for contraction. The motor end-plate, per se, was reported to remain intact in vitamin-E deficient rabbits (Anderson and Rickard, 1957).

**Effects of Inactivation on Skeletal Muscle**

In order to elucidate the functional relationship between nerve and muscle, many experiments have been carried out in denervated, regenerating, or embryological preparations (Orbeli, 1945; Diamond and Miledi, 1962;
Gutmann and Hnik, 1962; Gutmann, 1964; Drachman, 1967), and in muscles following nerve cross-union procedures (Buller et al., 1960). These approaches, although they are most valuable, seem to provide only indirect evidence.

During the last few years, several investigators indicated that much information would be gained if the neuromuscular impulse activity could be prevented for a prolonged period of time (Desmedt, 1959; Gutmann, 1963; Miledi, 1963; Katz and Miledi, 1967a). This should be achieved without section of the motor nerve and without any damage to the nerve and to the neuromuscular pathway. Since anatomical continuity of the motor nerve, including the contiguity of the neuromyal junction, are essential requisites for the physiological functioning of the muscle, it was felt that a non-destructive inactivation of the skeletal muscle by way of non-destructive nerve block, would provide valuable information concerning the trophic role of nerve impulses, per se.

Inactivation by De-afferentation and Tenotomy

Various "disuse" experiments, such as immobilization by spinal isolation and de-afferentation, or by tenotomy (Tower, 1937; Tower et al., 1941; Eccles, 1941b, 1944; Reid, 1941; Solandt et al., 1943; Ferguson et al., 1957; Johns and Thesleff, 1961; McMinn and Vrbová, 1967) have been explored in an endeavor to provide somewhat more direct data. These disuse experiments generally failed to reproduce the classical consequences of motor nerve section, though a certain degree of muscle atrophy almost invariably accompanied prolonged disuse by the foregoing
methods (Eccles, 1941b and 1944; Ferguson et al., 1957). It was reported that changes in electrical excitability of the muscle had occurred in certain disuse preparations (Reid, 1941; Desmedt, 1949), although the electrodiagnostic changes, generally, have been minimal or absent. Eccles (1941b) reported that, during prolonged disuse, electrical stimulation applied to the anterior tibialis muscle of the rabbit resulted in retardation of the muscle atrophy.

Inactivation by Motor Nerve Compression

An apparently simplified and possibly a more direct approach was employed by Denny-Brown and Brenner (1944) who produced conduction block of the motor nerve and corresponding muscle paralysis by graded compressions of the nerve with a tourniquet. Some more or less reversible damage to the nerve was produced by the ischemia from compression; some preparations, however, showed that the nerve endings were not altered in structure and there was no morphological change in the motor end-plates or in the muscle fibers. The authors concluded that fibrillation in the muscle was absent whenever the anatomical integrity of the nerve had been preserved.

Inactivation by Local Anesthetics

A reversible nerve conduction block by local anesthetic drip-infusion was used to produce muscle paralysis, by Gutmann and Zak (1961). The sciatic nerve of the rabbit was treated with a continuous novocaine-drip for 3 days; this "inactivation" of the muscle failed to reproduce the characteristic changes found in the 3-day surgical denervation controls.
A "denervation hypertrophy", however, was observed in the novocaine-block inactivated muscle; this change was attributed by the authors to a sudden slowing of the catabolic processes during the complete immobilization.

Recently, Sokoll et al. (1968) reported denervation-like effects in innervated skeletal muscle after prolonged but intermittent perimuscular treatment by a local anesthetic. The drug was injected subcutaneously, at about 12 hour intervals. The method was described as a local anesthesia "likely" to produce complete or partial immobilization of the extensor digitorum longus muscles only for about 10 hours of the day, for 7 days. At the same time, these investigators also injected the same anesthetic agent around the motor axon (at about the sciatic notch) in order to produce nerve conduction blockade. In the latter preparations, the muscle inactivation did not produce the denervation-like changes. The authors attributed the effects (i.e. the supersensitivity changes in the muscle treated directly) to a prolonged interference with the action of ACh, by the local anesthetic agent, on the blocked muscle membrane.

Although definitive data were lacking, both Denny-Brown and Brenner (1944) and Gutmann and Žák (1961) suggested that the trophic neural influence is most likely to be independent of nerve impulses. This suggestion or, at times, conclusion, has been voiced by an increasing number of investigators in the recent years (Luco and Eyzaguirre, 1955; Desmedt, 1959; Gutmann, 1963; Katz and Miledi, 1967a). The information obtained from the apparently "simplified" elimination of nerve impulses mentioned above (Denny-Brown and Brenner, 1944; Gutmann and Žák, 1961),
seems to be insufficient to support this as an acceptable conclusion. The inadequacy of information was caused by one or a combination of the following: the insufficient duration of nerve-impulse deprivation; the non-specific nature of the experimental design; or the introduction of artefacts or variables (such as some untoward side effects) produced by the methods used. In addition, none of the latter reports (Denny-Brown and Brenner, 1944; Gutmann and Zak, 1961; Sokoll et al., 1968) included information concerning electrophysiological assessment of the completeness of the muscle paralysis or of the effects of this paralysis on the electrophysiological and pharmacological parameters of the nerve or muscle, during or following such inactivity. In the work of Sokoll et al. (1968), for example, it was not made clear whether the muscle paralysis was complete and continuous, since the blockade was described as intermittent. In addition, this method is unlike a "simple" neuromuscular impulse elimination, because the possible toxic and/or metabolic effects of the drug, applied directly to the muscle, are not ruled out.

Summarizing, the effect of a prolonged and non-destructive nerve-impulse elimination, on the skeletal muscle, has not been determined. A long-lasting pharmacological blockade of the motor nerve, in order to produce a non-conducting axon and a completely inactivated muscle without irreversible damage to the structures involved, has not been available because the various agents acting on the motor axon were either too short acting or caused some degree of axon damage. A prolonged and reversible pharmacological nerve block was first reported by Robert and Oester (1964), using a local anesthetic agent applied in a special preparation.
CHAPTER II

STATEMENT OF THE PROBLEM

Changes in the electrophysiological and pharmacological characteristics of the mammalian skeletal muscle after nerve section have been well documented. Whether any or similar changes might occur after a prolonged non-destructive pharmacological nerve block, has not been determined.

Several investigators have compared the results of surgical denervation to the results of prolonged inactivation of the muscle by a variety of procedures (Tower et al., 1941; Eccles, 1944; Johns and Thesleff, 1961). A remarkable resemblance was found between the supersensitivity caused by surgical denervation and that which is caused by prolonged treatment with agents and/or methods which interfere with transmission (Thesleff, 1960a; Prabhu, Oester and Karczmar, 1962b). The points of similarity encouraged several investigators to extend the Law of Denervation as valid for all cases of prolonged interference with neuromuscular transmission, especially for cases of "pharmacological denervations" (Emmelin, 1961; Thesleff, 1960b).

In recent years, a great concern of some investigators about the role of a trophic neural influence on the muscle resulted in substantial research activities related to this problem, in the fields of neurophysiology, neuropharmacology and biochemistry (Gutmann, ed., 1962; Gutmann and Hnik, eds., 1963; Singer and Shade, eds., 1964). Increasing evidence and newer concepts point to the probability that neuromuscular impulses are not identical with, and can be separated adequately, from a
trophic mechanism without untoward effects on the muscle (Desmedt, 1959; Gutmann, 1963; Sharpless, 1964), although an active controversy continues (Thesleff, 1960b; Miledi, 1963).

Attempts to eliminate neuromuscular impulses, per se, have not been successful because of introduction of undesirable variables during the experiments (Tower et al., 1941; Denny-Brown and Brenner, 1944; Sokoll et al., 1968). Inactivation of the muscle by nerve conduction block was either short-lasting (Gutmann and Žak, 1961), or the method and data were equivocal (Sokoll et al., 1968). It was apparent that a long-lasting, non-destructive and reversible pharmacological nerve conduction blockade might provide valuable information regarding the effect(s) of prolonged absence of nerve impulses on the muscle.

Therefore, the purpose of this investigation was to deprive the skeletal muscle of the functions of its nerve supply for a prolonged period of time by non-destructive pharmacological means. This would produce only a "functional" change without significant "structural" alterations in the cellular architecture of the nerve or its relation and contiguity to its muscle. The aim was to examine whether a simple but complete reduction of nerve impulse traffic and, presumably, a consequent reduction in chemical transmitter activity would produce any of the classical signs of denervation sensitization. A preliminary work (Robert and Oester, 1964) indicated that a suitable experimental design might render this aim feasible.

With the application of a lidocaine-silicone polymer implant (see Methods) around the sciatic nerve of the rabbit, a continuous nerve
The conduction block can be maintained for up to 14 days. The absence of electrical activity in the muscle and a corresponding complete clinical muscle paralysis will be frequently monitored by appropriate clinical and electrophysiological methods. Selected EMG and electrodiagnostic studies will be done at frequent intervals during the nerve-impulse deprivation of the muscle and after reversal of the block with subsequent return of motor functions. During the prolonged and continuous neuromuscular inactivity of selected durations (while the block is still present), and also after reversal of the block, selected pharmacological agents are to be injected semi-close arterially in the muscle (in situ) to assess any possible changes indicative of pharmacological supersensitivity.

The findings will be discussed in the light of current neuropharmacological and neurophysiological knowledge. The data obtained are expected to help determine whether the excitatory and the trophic functions of the motor nerve may be "simply" dissociated without development of denervation changes and/or pharmacological supersensitivity.
CHAPTER III
MATERIALS AND METHODS

EXPERIMENTAL ANIMAL

New Zealand albino rabbits, weighing 1.85-2.20 kg, 7-8 weeks old, males and females, were used throughout the experiments. The animals were maintained on "Purina Rabbit Chow" supplemented daily with fresh lettuce, and water, ad lib. They were individually housed in steel cages located in well ventilated animal quarters. All experiments were conducted in accordance with current U.S. and International Conventions for animal care (see Appendix).

EXPERIMENTAL GROUPS

The animals were separated into five groups (designated by Roman numerals), four experimental groups and one intact control group. In the experimental groups, the animals were further distributed in "subgroups" for the following reason: The initial or primary experimental procedure was either surgical nerve section or the placement of an implant on the sciatic nerve, respectively (see below). The second experimental step or procedure was done after varying periods following the initiation of the primary procedure. A subgroup is designated by the Roman numeral of the group followed by the number of days, in parenthesis, giving the duration of the initial experimental procedure. For example, the primary experimental procedure in Group II animals was the placement of a lidocaine-silicone polymer implant on the sciatic nerve. The second experimental step, in this group, was the pharmacological studies. Accordingly, the designation
"II(8d)" refers to, or identifies, animals in a subgroup in which the neuromuscular impulse deprivation had been in effect for 8 days when the pharmacological studies were carried out. Or, "I(14d)" designates a subgroup of animals which were subjected to sciatic nerve section and the chronic denervation state had been in effect for 14 successive days when the pharmacological studies were done.

In the following, the procedures and the number of animals in each group are described, including the numerical distribution of the animals in the respective subgroups.

**Group I** — Chronic surgical denervation, followed by pharmacological studies at the end of the time lapse (number of days in parenthesis) in the respective subgroups:

I(3d) ----- 8 animals
I(6d) ----- 8 "
I(8d) ----- 8 " A total of
I(10d) ----- 6 " 42 animals
I(12d) ----- 6 "
I(14d) ----- 6 "

**Group II** — Prolonged pharmacological nerve conduction block on sciatic nerve produced by a lidocaine-silicone polymer implant (see Appendix). Pharmacological studies were carried out while the nerve-impulse deprivation was still in effect and without reversal of the block. Nerve impulses did not reach the anterior tibialis muscle prior to pharmacological sensitivity testing, which was done at the end of the time lapse (number of days in parenthesis) in each respective subgroup:

II(2d) ----- 4 animals
II(4d) ----- 4 "
II(6d) ----- 6 " A total of
II(8d) ----- 6 " 34 animals
II(10d) ----- 6 "
II(12d) ----- 4 "
II(14d) ----- 4 "
Group III -- Prolonged pharmacological nerve conduction block identical to the one employed in Group II. The block was reversed by removal of the implant at the end of the time lapse in the respective subgroups; pharmacological studies were done shortly after the complete recovery of neuromuscular functions.

III(8d) ----- 18 animals  
III(9d) ----- 8 "  
III(12d) ----- 3 "  
III(14d) ----- 3 "

A total of 32 animals

Group IV -- "Blank"-implant control by a silicone polymer implant, without lidocaine, on the sciatic nerve (see Appendix). The implant remained in place for at least 14 days, after which pharmacological studies were done. No subgroups; 20 animals.

Group V -- Intact (normal) control. 20 animals. In addition, the opposite limb of the experimental animals was also used as control.

SURGICAL PREPARATIONS

All animals undergoing surgical procedures (i.e. section of the sciatic nerve; the placement of the lidocaine-silicone polymer implant; or placement of a "blank" silicone polymer implant around the sciatic nerve) were premedicated with atropine sulfate (5.0 mg/kg, I.M.) and anesthetized with sodium pentobarbital (Nembutal; 22 mg/kg, I.V.) injected into the marginal vein of the ear. Details of the implantation technique are described in the Appendix.

Chronic Denervation. The right sciatic nerve was exposed and sectioned at about the midpoint of the thigh. Both free stumps were reflected and ligated. The incision was closed and the animal was allowed to recover from the general anesthesia.
Prolonged Pharmacological Nerve Conduction Block. The right sciatic nerve was carefully exposed and a lidocaine-silicone polymer implant ("perineural sleeve") was placed around the nerve trunk at about midpoint of the thigh. This implant was previously prepared as described in the Appendix. In some animals, after 8 days, a second implant (identical with the first) was used to replace the first one, employing the same procedure for implantation (the use of two successive implants is explained below).

The onset of the conduction block occurred in about 20-35 minutes after placement of the implant. The effectiveness of the block (i.e. whether nerve impulse conduction was completely blocked) was assessed by the absence of muscle action potentials when applying supramaximal single electrical stimuli (square wave pulses of 0.1 msec duration, once every 2.5 seconds, by a Grass S4G stimulator) to the sciatic nerve, proximal to the implant. Total absence of electrical activity in the anterior tibialis muscle, during nerve stimulation, was monitored through intramuscular bipolar electrodes with an electromyograph (EMG; Teca Corporation).

The perineural implant acted as a "slow-release" drug reservoir and produced a continuous conduction block of the sciatic nerve with a corresponding limb paralysis, lasting up to 14 days. The maximum time lapse, i.e. when the implant in situ became depleted of its drug reservoir, was about 9-9½ days. Attempts to use larger implants, or implants with higher lidocaine content, were unsuccessful.

In animals subjected to nerve conduction block for 9 days or less (see Experimental Groups), only one implant was used. In Group III (8d) animals, this implant was surgically removed at the end of the 8th day and
nerve-impulse conduction was allowed to resume. In Group III (9d) animals, the implant was not removed at all but was left in place and was allowed to become depleted of its drug reservoir, in situ. This occurred generally at the end of the 9th day or shortly after, and was signaled by the occurrence of electrical potentials in the anterior tibialis muscle and by the reoccurrence of motor and/or sensory functions in the previously paralyzed limb (see Results).

In animals with conduction block planned to last for more than 9 days, two successive implants were used in the following manner. At the end of the 8th day, the first implant was surgically removed and replaced by a "fresh" and identical lidocaine-polymer perineural sleeve. Continuity of the complete electrical silence in the anterior tibialis muscle was carefully monitored by EMG during the re-implantation procedure. The second implant then was left in place for additional periods of continuous blockade (see subgroups of Group II and III, with block duration over 9 days) and were still in situ when pharmacological studies terminated the experiments [Groups II(10d), II(12d), II(14d)]. In other animals [Groups III(12d) and III(14d)], the second implant was left in place until its drug reservoir became depleted which was assessed in a manner described in the foregoing, under Group III(9d).

"Blank"-Implant Control. A silicone polymer implant, without lidocaine, was placed around the sciatic nerve of animals in Group IV, in a manner identical to that described under "Prolonged Pharmacological Nerve Conduction Block" (see also Appendix).
EXAMINING PROCEDURES

All procedures of examination, described in the following section, were conducted daily on all animals in each group. More frequent examinations were done with particular groups of animals as specifically noted in the pertinent sections. In all animals, both the treated and the opposite anterior tibialis muscles were examined. The general examinations consisted of: 1) clinical neurological examination; 2) electromyographic examination; and 3) electrodiagnostic examination.

Clinical Neurological Examination

Diagnostic criteria for complete clinical paralysis were: a) complete foot drop; b) total absence of deep tendon reflexes; c) absence of grasp-reflex and of the "antigravity" ("righting")-reflex (the latter, when present, is observed as spreading of toes with eversion and dorsiflexion of the foot, during a sudden lowering of the animal, simulating a sudden fall); and d) total sensory anesthesia (i.e. no response to pin-prick, pinching, heat, or any other nociceptive stimuli). The presence of any measurable degree of motor or sensory signs or functions ruled out a complete clinical paralysis. In contrast, neurological findings were considered "normal" only when motor functions, including gait, and sensory perception, as tested by the aforementioned criteria, showed no deficit. The baseline or "model" for such normal neurological examination was that of the average intact control animal.
of the examining table and to have unrestricted movements of the limb for EMG recording.

After the ground electrode was attached, the bipolar recording electrode was inserted into the muscle under examination. The anterior tibialis muscle was examined with multiple insertions; in addition, the extensor digitorum longus muscles were occasionally examined with one or two electrode insertions. When the electrode was inserted into the muscle, occasionally a few motor unit potentials appeared in animals other than those with the complete nerve conduction block. When the animal was quiet, these potentials usually disappeared. Examination began with the "quiet" (or "zero activity") state of the muscle. Following this, muscle action potentials were observed and recorded during various conditions of muscle functions such as forced or reflex response to mechanical or nociceptive stimuli. Selected muscles were also examined under brief general anesthesia (diethyl ether by mask-inhalation) to detect possible fibrillation potentials or to analyze occasional equivocal muscle action potentials such as insertion activity.

Electrodiagnosis (EDX)

Instrumentation

EDX studies were performed with a constant-current impulse generator (MEDITRON) capable of delivering monophasic rectangular ("square wave") stimuli of 0.1 to 1000 msec duration, at variable frequencies. The current output of the generator was measured by a highly sensitive and well damped DC-milliammeter. The percutaneous stimulating electrode was a copper rod,
STRENGTH-DURATION CURVE is obtained by plotting the current(s) required to produce threshold contraction(s) against a series of decreasing durations of stimuli.

The procedure used to obtain the above parameters was carried out as follows: After depilating the skin over the test area of the limb under examination, the animal was positioned and restrained. The positive terminal of the stimulator was connected to the indifferent electrode, and the negative electrode was positioned over the motor point for percutaneous stimulation. The stimulator was set to deliver repetitive cathodal stimuli with a duration of 300 milliseconds, at 1 second intervals. The current control was advanced until a perceptible muscle contraction was seen and the stimulating electrode was carefully repositioned, in the motor point region, until the lowest threshold reading was obtained. For a complete S-D curve, the preceding step was repeated with successive decrements in stimulus duration, in the following order: 100, 60, 30, 10, 6, 3, 1, 0.6, 0.3, and 0.1 milliseconds. The interval between stimuli was kept at a constant 1 second.

REPETITIVE STIMULATION is an electrophysiological procedure to assess the responses of a muscle to repetitive electrical stimulation by determining the amount of current required, at predetermined frequencies, to produce sustained (tetanic) contractions. Uniform galvanic stimuli of short durations are used at selected intervals. In a normal muscle, the current values required for tetanic contractions remain essentially unchanged at all conventionally used frequencies. In contrast, in a chronically denervated
muscle progressively greater amounts of current are required as the frequency of stimulus decreases.

The following frequencies were used (pulse duration was kept at a constant 1 millisecond):

- 91 cycles/sec -- (pulse interval 10 msec)
- 166 cycles/sec -- (pulse interval 5 msec)
- 500 cycles/sec -- (pulse interval 1 msec)

**Conversion and Computation of EDX Data**

The electrical current required to excite the muscle at a specified duration or frequency was recorded daily from experimental and control anterior tibialis muscles. The thresholds of stimulation, at any given duration, shows a slight variability in the same normal muscle. To minimize the variability from one day to the next and from one rabbit to another, the strength-duration data obtained were standardized in order to allow comparison of these data. This was done by a quantitation method employed for EDX data handling by investigators in this laboratory (Fudema, 1958; Prabhu, 1962).

All points on a strength-duration curve were made a function of the rheobase for that particular curve. That is, all threshold values for the different durations of the stimuli for a given curve were divided by the rheobase for that curve. Rheobase now becomes unity and all other points on the curve are unity or greater. This quotient was designated as "threshold ratio" (TR). Threshold ratio may be defined as the magnitude of
current (in milliamperes) required to produce a rheobase-response, divided by the threshold current at 300 milliseconds duration:

\[
\text{TR} = \frac{\text{threshold current (in milliamperes) at a specific duration}}{\text{threshold current (in milliamperes) at "infinite" duration (300 msec)}}
\]

Fudema (1958) carried out an analysis of the variance between strength-duration data as "raw" data and following conversion of the raw data to threshold ratio values. On the basis of his findings, he concluded that conversion of strength-duration data to threshold ratios is a "valid procedure which renders the data more useful for comparison by direct observation as well as by statistical evaluation".

Strength-duration curves were obtained from the denervated anterior muscles before denervation ("zero day"), and on the 1st, 2nd, 4th, 6th, 8th, 10th, 12th, and 14th day following nerve section. For each one of these periodic examination days, the values for each duration of stimulus from each animal were converted to threshold ratios, averaged and the standard deviations were computed. In this manner, one composite S-D curve was obtained for each of the above examination days.

Strength-duration curves were obtained from the normal anterior tibialis muscles and from the muscles of lidocaine-treated and "blank"-control animals each day, beginning the day before implantation. For each particular group of animals, the values obtained for each stimulus duration on each particular day, were converted to threshold ratios. The ratios, for each stimulus duration and for each group were averaged and their standard deviations were computed. This resulted in a composite curve, for each particular group, each day. As the days went by and succeeding
examinations were made, the same process was repeated and the resulting curves were compared. It became apparent by the 14th day of examinations that the readings for the specific stimuli were essentially the same on any one day of the examination, in each particular group, and that the data could be averaged.

For that reason, the S-D curves for each one of these groups (Groups II, III, IV, and V), separately, were plotted in the following manner: In each particular group, all of the values obtained for each specific stimulus (from each one of the rabbits in this group) during the entire period of observation, were converted to threshold ratios, combined and averaged, and (for each stimulus duration) the standard deviations were computed. This method was employed earlier in this laboratory (Fudema, 1958; Prabhu, 1962), and was reported that this quantitation method was supported by the observation that, when individual S-D curve data from a group of normal rabbits were converted to threshold ratios and compared, no statistically significant difference was found between the composite curves and the individual curves obtained on the same day or on different days.

Rheobase values of the anterior tibialis muscles of the denervated, lidocaine-treated, "blank"-implant control, and intact control animals were averaged (separately for each group) on each day of the examination.

Chronaxie values, in all cases, were obtained from the plotted S-D curves. Values from a particular group of animals, for each particular day of measurement, were combined, and the average values computed. The standard deviations for the respective averages were then estimated.
Repetitive stimulation data, for each animal in any particular group, were converted to "repetitive-threshold ratios", which are the actual current values (in milliamperes) required for the tetanic contraction at the given frequency, divided by the amount of current (in milliamperes) required to produce twitch contraction at 300 msec. In other words, each actual value was divided by the rheobase assessed during that particular examination. Because it was found that the values obtained from the individual rabbits in each particular group, on any particular day of examination, were essentially alike, the threshold ratios in each group, on any particular day, were combined and averaged.

PHARMACOLOGICAL STUDIES

Surgical Preparation

The animal was anesthetized with Urethane (1.5 gm/kg, I.V.), injected into the marginal vein of the ear (25% Urethane in 0.9% saline). The injection of the anesthetic agent was done slowly, over a period of 5 to 15 minutes, under careful observation of respiratory and cardiac functions of the animal. For pre-anesthetic medication, atropine sulfate was used (1.5 mg/kg, intraperitoneally).

The appropriate areas of the skin were depilated. The skin was incised in the inguinal area for semi-close arterial cannulation for injection of pharmacological agents. At various points of the thigh and the leg, separate incisions were made in order to expose the knee, the ankle, and the anterior tibialis muscle, respectively.
The leg was fixed to prevent movement artifacts. Steel pins were placed through the distal condyles of the femur and/or the proximal head of the tibia (whichever seemed more accessible, or adequate in size and shape, for the purpose), and another one through the distal condyles of the tibia. The steel pins were connected to the supporting framework of clamps to immobilize the knee and the origin of the anterior tibialis muscle.

The muscle was exposed down to the ankle. At the anterior aspect of the ankle, the retinacula were cut, also some of the synovial tendon sheaths, in order to free the distal part of the anterior tibialis muscle and its tendon. The tendon was sectioned close to its point of insertion and was connected (by means of a strong and pre-stretched nylon thread) to a force transducer.

On the lateral side of the knee, the common peroneal nerve was exposed for stimulation. During the preparations, the nerve and the muscle were kept moist with physiological saline and kept at a constant 37°C temperature by means of a thermostatically regulated infrared lamp.

For drug injections, the femoral artery and its branches, in the inguinal region, were carefully exposed. A polyethylene catheter (#10) was inserted into a medial branch, the one that lies immediately distal to the issuance of the profunda femoris artery.

In an occasional animal, with anomalous vessels, the profunda femoris artery itself may have been cannulated when the collaterals assured proper blood supply to the muscle. Essentially all branches and collaterals were ligated except those perfusing the anterior tibialis muscle. Injection of dye into the cannula and the resulting limited staining assured that only the tibialis anterior muscle was perfused. This injection method
was an intermediate modification of two previously described methods, the close-arterial injection method of Brown et al. (1936) and the distant-arterial injection method of Zaimis (1951). It could be defined as a "semi-close" arterial or a "regional" arterial injection method.

All drugs were freshly prepared and all injecta were of 0.5 ml volume, followed immediately by 0.5 ml saline, both injected rapidly.

Instrumentation

For indirect stimulation, the common peroneal nerve was exposed and supramaximal single shocks were applied through a shielded Porter-type electrode, once every 2.5 seconds. For direct muscle stimulation, another Porter-type electrode was secured to the belly of the muscle. Supramaximal single shocks were applied, once every 2.5 seconds. The surrounding skin flaps were made to form a basin which was filled with mineral oil, equilibrated with 95% O₂ - 5% CO₂, and kept at a constant 37°C. In animals of Group II, where the pharmacological studies were carried out while the continuous conduction block was still in effect, supramaximal single stimuli (square wave pulses of 0.1 to 0.5 msec duration, from a Grass S4G stimulator) were applied to the sciatic nerve, proximal to the block (implant), and the absence of muscle action potentials in the anterior tibialis muscle was assessed by EMG. The sciatic trunk then was sectioned high in the thigh to prevent antidromic stimulation. Responses to injected pharmacological agents (ACh, SCh, and dTc) were assessed by recording the muscle twitches before, during, and after each particular injection while being continuously stimulated indirectly or directly. In the denervated controls only direct stimulation was used.
CHAPTER IV
EXPERIMENTAL RESULTS

CLINICAL NEUROLOGICAL OBSERVATIONS

Both limbs of animals in the experimental and control groups were examined daily for clinical neurological signs. Motor and sensory functions in the limbs of intact control rabbits, and those in the opposite (not treated) limb of experimental animals were used as criteria for normal neurological findings. Diagnostic criteria for complete clinical paralysis consisted of the neurological findings in the denervated limbs of Group I animals.

Group I -- Chronic Surgical Denervation

The surgically denervated limbs showed complete flaccid paralysis immediately upon recovery from surgical (general) anesthesia. There was a complete foot-drop, total absence of deep tendon reflexes, and absence of grasp-reflex and anti-gravity ("righting") reflex: i.e. there was no eversion and dorsiflexion of the foot with toe-fanning, during sudden lowering of the animal from table-to-floor (holding the animal by the loose skin folds of nape and back), simulating a fall. The gait was clearly monoplegic. Complete sensory deficit was unequivocal, as seen by the total absence of response to touch, pin-pricks, pinching, heat, and other nociceptive stimuli applied to the denervated limb; contralateral noxious stimulation produced no response in the paralyzed extremity.

These signs remained unchanged during the entire period of observation; in addition, a moderate degree of palpable muscle atrophy was noted,
generally, on the fourth day following denervation, progressing over the remainder of the observation period. Assessment of muscle atrophy by weight and/or volume of the muscle, or histopathological examination of the muscle, have not been included in this study.

**Group II and Group III -- Prolonged Neuromuscular Impulse Deprivation by Lidocaine-Silicone Polymer Implant**

These two groups are described together for the first phase of clinical observations, i.e. during the period(s) of "functional denervation" following placement of the axon-blocking implants. The second phase, i.e. reversal of the block, was done only in Group III.

The treated limb of animals in these groups showed complete flaccid paralysis at the time of recovery from general (surgical) anesthesia. A clearly visible foot-drop, and total absence of deep tendon reflexes and of the anti-gravity ("righting") reflex were observed. There was no grasp-response to plantar stroking. The gait was distinctly monoplegic (right hind limb). Sensory examination showed a complete absence of sensations to all modalities as assessed by the lack of movement in the paralyzed limb and by the absence of the usual manifestations of sensory appreciation by the animal (e.g. crying, eye-blinking, movements, etc.) to stimuli, including noxious ones. By this time, motor and sensory functions of the opposite (not treated) limb have returned and served as comparison.

The clinical paralysis remained unchanged during the various periods of observations, as presented in Table 1. No palpable muscle atrophy was noted during the course of the prolonged blockade or after reversal of the block, where this was done. Any indication of the presence of sensory or
motor functions, or the presence of palpable muscle atrophy, in the treated limb, excluded the animal from the study.

**Group III -- Reversal of Pharmacological Blockade**

The duration of continuous nerve conduction block and the corresponding complete clinical monoplegia was 8 days in Group III(8d). At the end of this period, the implant was surgically removed (see Materials and Methods), and neuromuscular impulse conduction was allowed to resume. Within 4 to 8 hours, following removal of the implant, a complete return of sensory and motor functions was observed in the previously paralyzed limbs. Clinical examination revealed no sensory or motor deficits, the foot-drop had fully recovered, all reflexes became normoactive and the animal's gait was within normal limits. An occasional animal continued to show a mildly paretic gait for as long as 14 to 16 hours following removal of the implant, although peripheral neurological findings indicated full recovery of neuromuscular functions. For example, the strength of the previously paralyzed muscle was equal to that of the opposite (control) muscle, as shown by the strength of resistance to passive movements, or by the force, speed, and range of motions in this leg when responding to nociceptive stimuli. Presumably, this occasional finding (of residual paresis), despite the absence of peripheral neurological deficits, may have been the result of a learned guarding by the animal (the manifestation of some residual conditioning effect), or was due to some other, transitory, nervous system influence, resulting from disuse. With passive exercise of this limb and by eliciting frequent reflex movements with repeated noxious stimuli, full recovery was complete within about 16 hours, and a normal gait was resumed.
In Group III(9d) animals, duration of continuous pharmacological nerve-impulse deprivation was over 9 days (range between 220–230 hours). In this subgroup, the implants were not removed surgically at the end of the 8th day. Instead, the blockade was allowed to continue until the implant was depleted (in situ) of its drug reservoir. This was signaled by the spontaneous re-occurrence of sensory and/or motor functions in the paralyzed limb, and/or by the appearance of electrical activity, by EMG. In order to detect resumption of neuromuscular activity without delay, clinical neurological examinations (also EMG monitoring) were done more frequently after the 8th day, at times as often as every 1 or 2 hours. The time of the last preceding examination during which the paralysis was still complete and/or electrical silence was still present, by EMG, was considered as the end of the period of continuous neuromuscular inactivity.

The time-course of complete resumption of normal clinical neurological functions (from the appearance of first signs until recovery was complete), as expected, was longer in these animals than in limbs from which the implants were surgically removed; it ranged between 8–18 hours. It is likely that the continued presence of the perineural implant (with its variable residue of lidocaine) slowed down recovery. Generally, at the end of about 18 hours, each animal regained normal sensory and motor functions in the previously paralyzed limb, with normal neurological findings equal to those described in the preceding section, for Group III(8d).

In Groups III(12d) and III(14d), duration of continuous pharmacological nerve-impulse deprivation was 12 and 14 days, respectively. This was
accomplished by the application of two successive implants, as described
in Materials and Methods. During placement of the second implant, EMG
monitoring insured continuity of the neuromuscular inactivity. The
second implant was not removed surgically but was left in place and the
animal was carefully observed for the re-occurrence of neuromuscular impulse
conduction (secondary to depletion of the local anesthetic agent in the
implant in situ), in a manner identical to that described in the foregoing
for Group III(9d). Furthermore, the frequency of clinical (and of EMG)
examinations was increased to 3 times per day, after placement of the
second implant (i.e. from the 9th day of the continuous blockade), and to
6 times per day beginning on the 12th day of blockade. The time of the
last examination, still showing complete paralysis, was selected as the
end of the total period of continuous nerve block. The course of recovery
was similar to that described for Group III(9d), above. A complete
recovery of sensory and motor functions was observed, including a normal
gait. No palpable muscle atrophy was noted.

Group IV -- "Blank"-Implant Control

Immediately upon recovery from general (surgical) anesthesia for
implantation of the "blank" polymer sleeve (without lidocaine) on the
sciatic nerve of these animals, the treated limb showed no clinical
neurological impairment. There was no foot-drop; the deep tendon reflexes,
the anti-gravity ("righting") reflex, and the grasp-reflex were present
and normoactive. Muscle strength was good with a normal range of motion;
the gait was within normal limits. These normal neurological findings
remained the same throughout the period of observations, for at least
14 days. In fact, a few animals were kept as long as 45 days after implantation (with the "blank" sleeve in situ) without showing any neurological deficit. No palpable muscle atrophy was noted.

**Group V -- Normal (Intact) Control**

The clinical neurological functions (and the parameters thereof) in the limbs of intact rabbits were selected as criteria for normal sensory, motor, and gait requirements, and used for comparison with the experimental limbs. In addition, the opposite limb of each treated animal was observed, for comparison, during clinical examinations. The criteria for a normal clinical neurological examination are described in Materials and Methods, under Examining Procedures.
### TABLE 1

**SUMMARY OF CLINICAL NEUROLOGICAL FINDINGS**

<table>
<thead>
<tr>
<th>Exper. Groups</th>
<th>No. of Animals</th>
<th>Duration of Paralysis (in days)</th>
<th>Clinical Neurological Findings - during Continuous Pharmacological Blockade or during the Control Procedures</th>
<th>Clinical Neurological Findings - following Reversal of Blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic Surgical Denervation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lidocaine Block</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr.II</td>
<td></td>
<td></td>
<td>Complete flaccid paralysis, identical clinical neurological findings as in Chronic Surgical Denervation, except no muscle atrophy here at any time.</td>
<td>No reversal done; pharmacological testing done while blockade is still in effect</td>
</tr>
<tr>
<td>II(2d)</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II(4d)</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II(6d)</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II(8d)</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II(10d)</td>
<td>6</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II(12d)</td>
<td>4</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II(14d)</td>
<td>4</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lidocaine Block</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gr.III</td>
<td></td>
<td></td>
<td>Complete flaccid paralysis, identical clinical neurological findings as in Chronic Surgical Denervation, except no muscle atrophy during prolonged blockade.</td>
<td>Full clinical recovery of sensory and motor functions shortly after elimination of blocking agent. No muscle atrophy at any time.</td>
</tr>
<tr>
<td>III(8d)</td>
<td>18</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III(9d)</td>
<td>8</td>
<td>9-9½</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III(12d)</td>
<td>3</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III(14d)</td>
<td>3</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>&quot;Blank&quot; Implant Control</strong></td>
<td>20</td>
<td>No Paralysis</td>
<td>Normal neurological findings throughout presence of &quot;blank&quot; implant in situ (up to at least 14 days)</td>
<td>-------</td>
</tr>
<tr>
<td>Gr.IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Intact) Control</td>
<td>20</td>
<td>--</td>
<td>Normal</td>
<td>-------</td>
</tr>
<tr>
<td>Gr.V</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ELECTROMYOGRAPHIC FINDINGS

Group I -- Chronic Surgical Denervation

Immediately upon recovery from surgical (general) anesthesia for neurotomy, there was a complete electrical silence, by EMG, in the anterior tibialis (and in the extensor digitorum) muscles of these animals. This silence, present at rest, remained unaltered upon passive movements of the limb or on homo- or contralateral nociceptive stimulation. On the second or third day, after nerve section, some irritation potentials and some excessive insertion activity were noticed. A few fibrillation potentials appeared on the third day and widespread fibrillation was unequivocally present on fourth day. These fibrillation potentials had an amplitude of approximately 50 uV to 350 uV, with a duration of 0.5 to 1.5 milliseconds; appeared as biphasic sharp spikes giving an auditory display which may be best described as sharp, high-pitched, galloping sounds or as a "shower of rain hitting the roof". Figure 1 presents electromyographic recordings from the anterior tibialis muscle of the rabbit, following chronic surgical denervation.

Group II and Group III -- Prolonged Neuromuscular Impulse Deprivation by Lidocaine-Silicone Polymer Implant

During the periods of "functional denervation" (for the various periods in the respective subgroups see Materials and Methods, under Experimental Groups, or Table 1), the electromyographic findings were essentially the same in these two groups and in their subgroups, throughout the observations. Immediately upon recovery from general (surgical) anesthesia for implantation, electromyographic examination of the blocked muscles produced complete
Electromyograms from the anterior tibialis muscle of the rabbit, following surgical denervation (Group I).

A -- After 2 days, electrical silence at rest.

B -- After 3 days, moderate "insertion activity" and few fibrillation potentials, with occasional positive sharp waves.

C -- After 4 days, widespread continuous fibrillation.
electrical silence on the oscilloscope. No spontaneous potentials occurred at rest, and no potentials could be evoked by passive movements of the paralyzed limb. Homo- or contralateral nociceptive stimulation of the animal left the silence on the EMG screen unaltered. No fibrillation potentials were found during any period of observation in any animal. There were no irritation potentials or excessive insertion activity noted. These findings were the same when the anterior tibialis muscle was examined by multiple electrode insertions or when the extensor digitorum muscles were tested with one or two insertions. The auditory display was equally silent. Figure 2 shows electromyographic recordings from the anterior tibialis muscle of rabbits after various durations of neuromuscular impulse deprivation.

**Group III -- EMG Findings After Reversal of Block**

After various durations of pharmacological nerve block (for durations, see Clinical Neurological Observations, under Group III -- Reversal of Pharmacological Blockade) and following either surgical removal of the implant or depletion of drug reservoir from the implant in situ, a few normal-type motor unit potentials occurred within a few hours. These increased in number and reached a full interference pattern (with a corresponding clinical recovery) within 4 to 18 hours, depending upon the subgroup tested. No fibrillation potentials or any other abnormal EMG potentials were found in animals of any subgroup, at any time, during the inactivation or following complete recovery. No excessive insertion activity was noted. In addition, repetitive nerve stimulation produced no change (increase or decrease) in the amplitude of muscle potentials.
Electromyograms from the anterior tibialis muscle of the rabbit following various durations of neuromuscular impulse deprivation by lidocaine-silicone polymer implant around the sciatic nerve (Group III).

A -- Two hours after implantation, complete electrical silence at rest, on passive movement of the limb, or on painful stimulation of the limb.

B -- After 6 days, continued (uninterrupted) electrical silence.

C -- After 14 days, no change in electrical silence.
Figure 3 shows electromyographic recordings from the anterior tibialis muscle of the rabbit following EMG recovery after removal (or depletion in situ) of the blocking implant.

Group IV -- "Blank"-Implant Control

Immediately upon recovery from general (surgical) anesthesia for implantation of the "blank" (without lidocaine) perineural sleeve, electromyographic recordings from the treated anterior tibialis muscles were identical to those obtained from normal controls. No EMG abnormalities were found during the periods of observation (at least 14 days and occasionally much longer), i.e. during the time the implant was in situ. There was a complete electrical silence at rest; few or many normal motor unit potentials, with characteristic parameters, appeared on passive movements of the treated limb or upon reflex movements of the limb while responding to nociceptive stimuli (all findings were the same as in normal controls; see next paragraph).

Group V -- Normal (Intact) Control

In the intact (normal) anterior tibialis muscle of the rabbit, electromyographic recordings produce an electrical silence at complete rest (isoelectric baseline, with presence of EMG noise, if any, which in this study was below 5 microvolts). Upon mild passive or with slight voluntary movement of the animal, a few normal motor unit potentials are produced. When muscle contraction is maximal, as in the case of reflex movements (e.g. strong withdrawal response due to homo- or contralateral nociceptive stimulation), an "interference pattern" is produced on the
Electromyograms from the same anterior tibialis muscle of the rabbit in Figure 2, following "reversal" of the block by removal of the implant (Group III).

A -- After 12 hours, few normal motor unit potentials on passive movement of the limb.

B -- After 16 hours, many motor unit potentials on forceful contraction, secondary to withdrawal response to irritation.

C -- After 4 days, electrical silence at rest. No fibrillation potentials seen. (Same findings of silence on days 1, 2, and 3).
oscilloscope, from summation of many motor units. No abnormal EMG potentials, such as fibrillation potentials, are seen even under general anesthesia. Figure 4 presents electromyographic recordings from the normal anterior tibialis muscle of the rabbit under the conditions described in the foregoing. Records from "blank"-implant treated (on the sciatic nerve) muscles were essentially identical to records obtained from normal controls under corresponding experimental conditions.

Table 2 presents a summary of electromyographic findings under the various experimental conditions in the respective groups.
FIGURE 4

Electromyograms from intact (normal control) anterior tibialis muscle of the rabbit (Group V). Identical records were obtained from muscles with "blank"-implants on the sciatic nerve (Group IV).

A -- At rest, complete electrical silence (essentially isoelectric baseline).

B -- Few normal motor unit potentials with mild passive or volitional movements of the limb.

C -- Interference pattern from summation of many motor units upon maximal contraction due to contralateral painful stimulation.
### TABLE 2

**SUMMARY OF ELECTROMYOGRAPHIC FINDINGS**

<table>
<thead>
<tr>
<th>Exper. Groups</th>
<th>EMG During Muscle Inactivation As Compared to Controls</th>
<th>EMG Following Reversal of Block As Compared to Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.II &amp; Sub.-groups</td>
<td>Complete electrical silence, immediately upon lidocaine implant placement, at rest or upon passive movements. Remained the same during all blocking periods. No fibrillation potentials or excessive insertion activity.</td>
<td>No reversal of block done; pharmacological studies carried out while block was still in effect.</td>
</tr>
<tr>
<td>Gr.III &amp; Sub.-groups</td>
<td>Complete electrical silence, immediately upon lidocaine implant placement, at rest or upon passive movements. Silence remains throughout the various inactivation periods. No fibrillation potentials or excessive insertion activity at any time.</td>
<td>Rapid return of few normal motor units shortly after removal of block. Interference pattern could be achieved on forceful contraction by maximum 18 hrs. after reversal. No fibrillation potentials or excessive insertion activity at any time. Identical to EMG findings in normal controls.</td>
</tr>
<tr>
<td>Gr.I</td>
<td>Electrical silence immediately after nerve section. In 3 days, some &quot;insertion activity&quot; and few fibrillation potentials, plus rare positive sharp waves seen. Widespread fibrillation after 4 days.</td>
<td>No reversal.</td>
</tr>
<tr>
<td>Gr.IV</td>
<td>Normal EMG findings during presence of &quot;blank&quot;-implant on the sciatic nerve, through entire procedure. Identical to EMG in normal control.</td>
<td>No reversal.</td>
</tr>
</tbody>
</table>
ELECTRODIAGNOSTIC (EDX) FINDINGS

Definitions of the EDX procedures and methods employed are described in Materials and Methods, together with explanation for the conversion and computation of EDX data.

Rheobase

The average rheobase values of the lidocaine block anterior tibialis muscles (Groups II and III) remained between 0.80 and 1.00 milliampere, during the entire duration of complete nerve-impulse deprivation. These values correspond to rheobase values generally known for normal rabbit anterior tibialis and to normal control values obtained in this study. Rheobase values in "blank"-implant control muscles were essentially within the same limits of those in normal (intact) controls. In contrast, the rheobase values in the surgically denervated anterior tibialis muscles became elevated to about 1.29 (± S.D.) by the end of the second postoperative day and then began to decrease progressively and fell to 0.29 (± S.D.) milliampere by the eighth, and to 0.21 (± S.D.) milliampere by the fourteenth postoperative day. Comparison of rheobase values in the treated and in the control muscles are presented in Table 3 and plotted in Figure 5.

Chronaxie

Chronaxie values were calculated from the respective strength-duration curves. Values obtained from the individual rabbit muscles in each group were compared and averaged for each day of examinations. In the anterior tibialis muscles, treated by lidocaine-silicone polymer nerve implant, the chronaxie values remained below 1.0 millisecond during the entire duration
### TABLE 3

**COMPARISON OF AVERAGED RHEOBASE VALUES WITH STANDARD DEVIATIONS (in milliamperes)**

<table>
<thead>
<tr>
<th>Days After Nerve Section or Implantation</th>
<th>Chronic Surgical Denervation (Group I)</th>
<th>Lidocaine Block (Group II)</th>
<th>Lidocaine Block (Group III)</th>
<th>&quot;Blank&quot; Implant (Group IV)</th>
<th>Intact Control (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;zero&quot; day (pre-procedure)</td>
<td>.87 ± .15 (42)*</td>
<td>.83 ± .16 (34)*</td>
<td>.80 ± .17 (32)*</td>
<td>.81 ± .14 (20)*</td>
<td>.84 ± .16 (20)*</td>
</tr>
<tr>
<td>1</td>
<td>.86 ± .19 (42)</td>
<td>.83 ± .13 (34)</td>
<td>.82 ± .14 (32)</td>
<td>.80 ± .17</td>
<td>.80 ± .11</td>
</tr>
<tr>
<td>2</td>
<td>1.29 ± .13 (42)</td>
<td>.81 ± .14 (34)</td>
<td>.80 ± .16 (32)</td>
<td>.83 ± .11</td>
<td>.82 ± .14</td>
</tr>
<tr>
<td>4</td>
<td>.99 ± .14 (34)</td>
<td>.85 ± .14 (30)</td>
<td>.84 ± .12 (32)</td>
<td>.82 ± .14</td>
<td>.80 ± .13</td>
</tr>
<tr>
<td>6</td>
<td>.76 ± .10 (34)</td>
<td>.82 ± .09 (26)</td>
<td>.84 ± .15 (32)</td>
<td>.83 ± .13</td>
<td>.80 ± .11</td>
</tr>
<tr>
<td>8</td>
<td>.29 ± .09 (26)</td>
<td>.84 ± .09 (20)</td>
<td>.80 ± .13 (32)</td>
<td>.83 ± .15</td>
<td>.85 ± .14</td>
</tr>
<tr>
<td>9</td>
<td>.24 ± .07 (18)</td>
<td>.82 ± .12 (14)</td>
<td>.86 ± .14 (14)</td>
<td>.81 ± .14</td>
<td>.82 ± .13</td>
</tr>
<tr>
<td>12</td>
<td>.22 ± .11 (12)</td>
<td>.80 ± .13 (8)</td>
<td>.82 ± .14 (6)</td>
<td>.84 ± .14</td>
<td>.83 ± .15</td>
</tr>
<tr>
<td>14</td>
<td>.21 ± .08 (6)</td>
<td>.81 ± .11 (4)</td>
<td>.83 ± .12 (3)</td>
<td>.82 ± .15</td>
<td>.82 ± .14</td>
</tr>
</tbody>
</table>

* (Number of animals)
RHEOBASE values in the chronically denervated, lidocaine-silicone polymer blocked and control anterior tibialis muscles of the rabbit. Each point on each curve represents the average value obtained from the number of animals in the particular group or subgroup, as listed under Experimental Animals (in Materials and Methods); the averaged values, with standard deviations, are listed in Table 1.
of complete nerve-impulse deprivation (in Groups II and III, and their subgroups). There was essentially no difference in these values among the subgroups. This corresponds to normal values known for the anterior tibialis muscle of the rabbit and to values assessed in normal (intact) and in "blank"-implant treated control muscles in our studies. The frequency of EDX examinations and the values obtained in the blocked muscles ruled out possible short-term changes, such as a temporary reaction of degeneration, that may have been reversed and missed. In contrast, chronaxie values in the surgically denervated anterior tibialis muscles showed an average ten-fold increase by the fourth day following nerve section, i.e. the pre-operative chronaxie value of 0.38 (± S.D.) increased to 3.87 (± S.D.) by the fourth postoperative day, and had continued to increase progressively afterward, reaching a value of 23.75 (± S.D.) on the fourteenth day of chronic denervation state: a seventy-five fold increase. Comparison of computed chronaxie values in the treated and control muscles are presented in Table 4 and the values are plotted in Figure 6.

Strength-Duration Curves

Threshold currents required to provide distinct and reproducible minimal end-point contractions were determined for each of the following durations of stimuli: 300, 100, 60, 30, 10, 6, 3, 1, 0.6, 0.3, and 0.1 milliseconds. The interval between stimuli was kept at a constant 1000 milliseconds. The method of conversion and computation of data is described in Materials and Methods.

The S-D curves assessed in muscles that have been totally deprived of nerve (neuromuscular) impulses continuously for periods up to 14 days,
<table>
<thead>
<tr>
<th>Days After Nerve Section or Implantation</th>
<th>Chronic Surgical Denervation (Group I)</th>
<th>Lidocaine Block (Group II)</th>
<th>Lidocaine Block (Group III)</th>
<th>&quot;Blank&quot; Implant (Group IV)</th>
<th>Intact Control (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;zero&quot; day (pre-procedure)</td>
<td>.38 ± .08 (42)*</td>
<td>.37 ± .11 (34)*</td>
<td>.43 ± .14 (32)*</td>
<td>.39 ± .08 (20)*</td>
<td>.41 ± .11 (20)*</td>
</tr>
<tr>
<td>1</td>
<td>.42 ± .09 (42)</td>
<td>.40 ± .12 (34)</td>
<td>.41 ± .09 (32)</td>
<td>.42 ± .11</td>
<td>.43 ± .11</td>
</tr>
<tr>
<td>2</td>
<td>1.88 ± .46 (42)</td>
<td>.39 ± .13 (34)</td>
<td>.40 ± .11 (32)</td>
<td>.38 ± .09</td>
<td>.39 ± .11</td>
</tr>
<tr>
<td>4</td>
<td>3.87 ± .93 (34)</td>
<td>.41 ± .15 (30)</td>
<td>.43 ± .11 (32)</td>
<td>.41 ± .13</td>
<td>.41 ± .10</td>
</tr>
<tr>
<td>6</td>
<td>5.71 ± 1.03 (34)</td>
<td>.41 ± .14 (32)</td>
<td>.40 ± .13 (32)</td>
<td>.40 ± .14</td>
<td>.42 ± .13</td>
</tr>
<tr>
<td>8</td>
<td>14.62 ± 2.09 (34)</td>
<td>.42 ± .09 (20)</td>
<td>.39 ± .12 (32)</td>
<td>.42 ± .09</td>
<td>.39 ± .10</td>
</tr>
<tr>
<td>9</td>
<td>18.47 ± 3.87 (18)</td>
<td>.39 ± .10 (14)</td>
<td>.42 ± .09 (14)</td>
<td>.39 ± .08</td>
<td>.38 ± .09</td>
</tr>
<tr>
<td>12</td>
<td>21.85 ± 4.92 (12)</td>
<td>.43 ± .14 (8)</td>
<td>.40 ± .11 (6)</td>
<td>.41 ± .12</td>
<td>.43 ± .14</td>
</tr>
<tr>
<td>14</td>
<td>23.75 ± 5.24 (6)</td>
<td>.40 ± .11 (4)</td>
<td>.42 ± .08 (3)</td>
<td>.43 ± .12</td>
<td>.40 ± .08</td>
</tr>
</tbody>
</table>

* (Number of animals)
FIGURE 6

Averaged CHRONAXIE values obtained in the surgically denervated, lidocaine-silicone polymer blocked, and control anterior tibialis muscles of the rabbit. Each point on each curve represents the average value obtained from the number of animals in the particular group or subgroup, as listed under Experimental Animals (in Materials and Methods); the averaged values, with standard deviations, are listed in Table 1. "DAYS" indicate the number of days following nerve section or initiation of the block.
(Groups II and III, and their subgroups) showed no significant change, at any time, from the classical shape of asymptotic hyperbole curve known for normal muscles, and were essentially identical to S-D curves obtained in normal control muscles in this study. In contrast, there was a significant alteration in the slope and in the position of S-D curves computed from data assessed in the chronically denervated anterior tibialis muscle. The change in the latter muscles became manifested as early as the second postoperative day. Comparison of strength-duration data of the treated and of the control muscles are presented in Table 5 and are plotted on semi-log paper in Figures 7 and 8.

**Repetitive Stimulus Curves**

Responses to repetitive electrical stimulation at 91, 166, and 500 cps frequencies were assessed and converted to repetitive threshold ratios, as described under Methods. The averaged computed values estimated in the lidocaine blocked, the surgically denervated, and in the control groups are presented in Table 6 and are graphically plotted in Figure 9. These values were found to remain essentially unchanged, at all three frequencies, during the various periods of nerve-impulse deprivation in the anterior tibialis muscles of lidocaine block treated animals. These findings fully correspond to the values of Repetitive Stimulation Indices assessed in the "blank"-implant controls and in the intact anterior tibialis muscles. In contrast, in the surgically denervated anterior tibialis muscles, progressively greater amounts of current were required as frequency of stimulus was decreased. Furthermore,
the current required for tetanic contractions, at all three frequencies used, increased progressively, pari passu, during the 14 days of the postoperative course.

Table 7 summarizes the electrodiagnostic findings.
TABLE 5

STRENGTH-DURATION DATA

Averaged Threshold Ratios with Standard Deviations (±S.D.)

<table>
<thead>
<tr>
<th>Duration (msec)</th>
<th>Controls (Intact &amp; &quot;Blank&quot;)†</th>
<th>Lidocaine Block</th>
<th>Chronic Surgical Denervation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-14†(40)*</td>
<td>1-14†(66)*</td>
<td>Days Following Nerve Section Or Implantation</td>
</tr>
<tr>
<td>300</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>100</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>60</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>30</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00 ±.02 ±.22 ±.16</td>
</tr>
<tr>
<td>10</td>
<td>1.00 ±.03 ±.06 ±.21</td>
<td>1.06 ±.07 ±.22 ±.16</td>
<td>1.39 ±.19 ±.31 ±.31 ±.52 ±.39</td>
</tr>
<tr>
<td>6</td>
<td>±.14 ±.18 ±.24 ±.39</td>
<td>±.16 ±.22 ±.39 ±.51</td>
<td>1.65 ±.38 ±.65 ±.56 ±.62</td>
</tr>
<tr>
<td>3</td>
<td>1.21 ±.16 ±.21 ±.23 ±.31 ±.41 ±.71</td>
<td>±.19 ±.38 ±.63 ±.91 ±.98 ±.98</td>
<td>2.17 ±.31 ±.71 ±.98 ±.98 ±.98</td>
</tr>
<tr>
<td>1</td>
<td>1.49 ±.24 ±.28 ±.39 ±.43 ±.53 ±.71</td>
<td>±.24 ±.39 ±.53 ±.71 ±.98 ±.98 ±.98</td>
<td>4.17 ±.43 ±.53 ±.71 ±.98 ±.98 ±.98</td>
</tr>
<tr>
<td>0.6</td>
<td>±.27 ±.29 ±.32 ±.43 ±.53 ±.71</td>
<td>±.28 ±.39 ±.53 ±.71 ±.98 ±.98 ±.98</td>
<td>5.39 ±.39 ±.53 ±.71 ±.98 ±.98 ±.98</td>
</tr>
<tr>
<td>0.3</td>
<td>2.19 ±.38 ±.54 ±.63 ±.82 ±.98</td>
<td>±.36 ±.46 ±.63 ±.82 ±.98 ±.98 ±.98</td>
<td>9.22 ±.39 ±.53 ±.71 ±.98 ±.98 ±.98</td>
</tr>
<tr>
<td>0.1</td>
<td>3.34 ±.35 ±.54 ±.71 ±.98 ±.98</td>
<td>±.55 ±.55 ±.55 ±.55 ±.55 ±.55 ±.55</td>
<td>17.82 ±.55 ±.55 ±.55 ±.55 ±.55 ±.55 ±.55</td>
</tr>
</tbody>
</table>

SEE NEXT PAGE FOR LEGENDS
LEGENDS

TABLE 5 (continued)

LEGENDS FOR STRENGTH-DURATION DATA

(See TABLE 5 on the preceding page)

LEGENDS

* (Number of animals) -- For "lidocaine block" see Groups II and III and subgroups, in Materials and Methods, for number in each subgroup.

† No significant difference was found between intact control and "blank"-implant control animals, at any time during the 14 days.

‡ Average daily threshold ratios were essentially the same for each day of the 14 day period, in all subgroups.
Semilog plot of STRENGTH-DURATION CURVES in the surgically denervated, lidocaine-silicone polymer blocked, and control anterior tibialis muscles of the rabbit, representing 1-8 days of the respective procedures. Each point on each curve represents the average value obtained from the number of animals in the particular group or subgroup, as listed under Experimental Animals (in Materials and Methods); the averaged values, with standard deviations, are listed in Table 1.

Figure 8 presents the S-D Curves obtained on 9-14 days of the respective procedures.

*Strength-Duration Curves were essentially unchanged during the periods of 1-14 days.
STRENGTH-DURATION CURVES obtained on days 9-14 of the respective procedures (same preparations as Figure 7). Each point on each curve represents the average value obtained from the number of animals in the particular group or subgroup, as listed under Experimental Animals (see Materials and Methods); the averaged values, with standard deviations, are listed in Table 1.

**FIGURE 8**
(Companion Curve of Figure 7)
TABLE 6

AVERAGED REPETITIVE STIMULATION INDICES WITH STANDARD DEVIATIONS

(Tetanus Threshold Ratios)

<table>
<thead>
<tr>
<th>Frequency (cps)</th>
<th>Intact Control</th>
<th>&quot;Blank&quot; Control</th>
<th>Lidocaine Block</th>
<th>Chronic Surgical Denervation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Days Following Nerve Section or Implantation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-14†(20)*</td>
</tr>
<tr>
<td>91 cps</td>
<td>1.53 ±.22</td>
<td>1.56 ±.24</td>
<td>1.55 ±.25</td>
<td>1.49 ±.27</td>
</tr>
<tr>
<td>166 cps</td>
<td>1.50 ±.31</td>
<td>1.47 ±.29</td>
<td>1.52 ±.22</td>
<td>1.53 ±.34</td>
</tr>
<tr>
<td>500 cps</td>
<td>1.69 ±.29</td>
<td>1.72 ±.27</td>
<td>1.74 ±.32</td>
<td>1.73 ±.37</td>
</tr>
</tbody>
</table>

* (Number of animals) -- For number of animals in the respective subgroups of Groups II and III, see Materials and Methods.

† Essentially the same on any day during the nerve-impulse deprivation, up to 14 days, in the lidocaine treated animals. Unchanged in "blank" controls, as in intact controls.
REPETITIVE STIMULUS CURVE data assessed in the surgically denervated, lidocaine-silicone polymer blocked, and control anterior tibialis muscles of rabbits. Each point on each curve represents the average value obtained from the number of animals in the particular group or subgroup, as listed under Experimental Animals (in Materials and Methods); the averaged values, with standard deviations, are listed in Table 1. "DAYS" indicate the number of days following nerve section or initiation of the block.

*Essentially the same at all 3 frequencies.
**TABLE 7**

**SUMMARY OF ELECTRODIAGNOSTIC FINDINGS**

<table>
<thead>
<tr>
<th>Experimental Procedure</th>
<th>Chronic Surgical Denervation (Group I)</th>
<th>Prolonged Nerve-Impulse Deprivation By Lidocaine Block (Groups II &amp; III)</th>
<th>Intact and &quot;Blank&quot; Implant Controls (Groups IV &amp; V)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RHEOBASE</strong></td>
<td>Elevated to 1.29 m.amp in 2 days; falls below normal by the 6th day; progressively falls to 0.21 m.amp by the 14th day.</td>
<td>Corresponds to normal values: between 0.8-1.0 m.amp. Remains unchanged during various periods of block, up to 14 days.</td>
<td>Normal, 0.8-1.0 m.amp. No change with time.</td>
</tr>
<tr>
<td><strong>CHRONAXIE</strong></td>
<td>Elevated soon after nerve section; ten-fold increase by the 4th postop. day; 75-fold increase on the 14th postop. day.</td>
<td>Remains below 1.0 msec. (corresponds to normal values) during periods of block, up to 14 days; no change with time.</td>
<td>Normal; no change with time.</td>
</tr>
<tr>
<td><strong>S-D CURVES</strong></td>
<td>Marked alteration in position and slope, starting on the 2nd postop. day. Stimulation at lesser duration requires increased current flow.</td>
<td>Corresponds to normal curves: the classical asymptotic hyperbole shape. No change at any time during periods of block.</td>
<td>Normal: classical hyperbole. No change with time.</td>
</tr>
<tr>
<td><strong>REPETITIVE STIMULUS CURVES</strong></td>
<td>Marked increase in threshold and change in pattern with different frequencies.</td>
<td>Values about the same at all 3 frequencies: corresponding to normal. No change with time.</td>
<td>Normal pattern and thresholds. No change with time.</td>
</tr>
</tbody>
</table>
PHARMACOLOGICAL FINDINGS

Semi-close arterial injections of selected pharmacological agents into the treated and the control anterior tibialis muscles, in situ, were done as described in Methods.

Sensitivity to Acetylcholine (ACh)

Group I -- Chronic Surgical Denervation

In this group, neuromuscular transmission was absent from the third day on, following nerve section. Accordingly, ACh injections were done during direct stimulation of the muscle (cf. Methods). Marked increase in sensitivity to ACh was unequivocally present after the third day post-denervation. Threshold dose of ACh, at this time, was already an average of 0.40 ug (range 0.30-0.55 ug), and the "massive" dose of ACh was an average 8.0 ug (range 6.5-9.0 ug). The supersensitivity (to ACh) change in the denervated muscles was fully manifest and at its maximum on the sixth day and afterward following nerve section. In all subgroups which were tested after the sixth day or later, there was little variation in the threshold dose which was an average 0.10 ug (range 0.08-0.13 ug). The "massive" dose of ACh was an average 4.0 ug (range 2.5-4.4 ug). Figure 10 shows responses to injected doses of ACh in denervated anterior tibialis muscles, 14 days following nerve section. The response to 4.0 ug of ACh shows a marked contracture with a greatly depressed response to electrical stimulation during the contracture. This finding in the denervated muscles corresponds to the classical observation noted by Dale and Gasser (1926) and to data reported by Brown (1937).
FIGURE 10

Polygraph recordings of mechanical responses to semi-close arterially injected ACh in the chronically denervated anterior tibialis muscles of rabbits (14 days after nerve section), during direct electrical stimulation. Supramaximal twitches were elicited once every 2.5 seconds.

A -- At the arrow, a threshold dose of 0.1 ug ACh was injected.
B -- At the arrow, a "massive"-dose of 4 ug ACh was injected.
Group II and Group III -- Prolonged Pharmacological Nerve Conduction Block

As described in Methods, the pharmacological studies were carried out in Group II while the uninterrupted neuromuscular impulse deprivation was still in effect; in Group III, these studies were done following "reversal" of the block after the various periods of continuous neuromuscular impulse inactivity. There was no change in the sensitivity to ACh in any one subgroup of Groups II and III, whether the muscles were tested after 2 days or after 14 days of the block, or after any periods in between. Figure 11 shows responses of anterior tibialis muscles to injected ACh after 14 days of block and while the block was still, uninterruptedly, present (Group II). The average threshold dose of ACh was 50 ug (range 40-52 ug). The average "massive" dose of ACh was 400 ug (range 350-500 ug). These doses were essentially within the range of doses used in the controls and the responses were essentially identical in character, and no significant difference was seen in the drug thresholds or muscle responses whether drug injections were done under indirect or direct electrical stimulation.

Group IV -- "Blank"-Implant Control, and
Group V -- Intact (Normal) Control

Identical responses were obtained to injected ACh in these two control groups. The threshold dose of ACh was an average 50 ug (range 35-55 ug); the average "massive" dose of ACh was about 400 ug (range 350-500 ug). Figure 12 shows responses of "blank"-implant control muscles (14 days after the "blank" implant had been in situ), to test doses of ACh. Figure 13 shows corresponding responses of intact control anterior tibialis muscles.
FIGURE 11

Polygraph recordings of mechanical contractions in the anterior tibialis muscles of rabbits, as responses to semi-close arterially injected ACh. Fourteen days of continuous neuromuscular impulse deprivation by a lidocaine-silicone polymer implant on the sciatic nerve (in situ), and while the nerve-block was still, uninterruptedly, present. Injections were done during indirect electrical stimulation; supramaximal twitches were elicited once every 2.5 seconds.

A -- At the arrow, a threshold dose of 50 ug of ACh was injected.
B -- At the arrow, a "massive"-dose of 400 ug of ACh was injected.
Polygraph recordings of mechanical contractions in the anterior tibialis muscles of rabbits, as responses to semi-close arterially injected ACh. In the above preparations, "blank" silicone polymer implants had been around the sciatic nerves, in situ, for 14 days. Injections were done during indirect electrical stimulation; supramaximal twitches were elicited once every 2.5 seconds.

A -- At the arrow, a threshold dose of 50 ug ACh was injected.

B -- At the arrow, a "massive"-dose of 400 ug ACh was injected.
Polygraph recordings of mechanical contractions in the anterior tibialis muscles of untreated (normal) control rabbits. Injections were done during indirect electrical stimulation; supramaximal twitches were elicited once every 2.5 seconds.

A -- At the arrow, a threshold dose of 50 ug ACh was injected.

B -- At the arrow, a "massive"-dose of 400 ug ACh was injected.
Responses to Injected Succinylcholine and d-Tubocurarine

A brief examination of the effects of selected neuromuscular blocking agents on the nerve-impulse deprived muscles was done by semi-close arterial injections (in situ) of a depolarizing drug, SCh, and of a non-depolarizing drug, dTc, during indirect electrical stimulation. In the denervated muscles, these drugs were not used because indirect electrical stimulation was ineffective, from the 3rd day on after nerve section, indicating the cessation of neuromuscular transmission.

In the lidocaine nerve-block inactivated muscles, however, prior to the injections of the neuromuscular blockers, indirect electrical stimulation resulted in muscle twitches, essentially identical to those in the "blank"-implant and normal controls. Following injections of the respective drugs, the responses to identical doses were also remarkably similar in the lidocaine inactivated and in the control muscles. These drug effects were assessed by the type (i.e. stimulation and/or depression of the muscle twitch) and the magnitude of neuromuscular blockade, and by the time-course of recovery of the muscle twitch to an approximate pre-injection level (under indirect stimulation). No attempt was made to measure synaptic delay or to assess the actual site of action of either drug.

The exact site of action of SCh and dTc (i.e. whether they act pre- or postsynaptically, or at both sites) is not entirely clear. This brief examination of their effects is expected to give a gross assessment of the functional status of the neuromyal junction, especially in comparison to "blank"-implant and control effects.
Figures 14 and 15 show a comparison of responses to semi-close arterially injected 10 ug of SCh (Figure 14) and of 20 ug of dTc (Figure 15). Responses to larger doses of SCh or dTc, respectively, were essentially the same in the three muscles compared.

Table 8 summarizes the pharmacological findings.
Polygraph recordings of mechanical contractions; comparison of responses to semi-close arterially injected Sch (at the arrows) in the treated and control anterior tibialis muscles of rabbits (in situ), during indirect electrical stimulation. Supramaximal twitches were elicited once every 2.5 seconds.

A -- Normal (intact) control.
B -- "Blank"-implant control (implant in place for 14 days).
C -- Lidocaine-silicone polymer treated sciatic nerve, on the 14th day of continuous lidocaine block.
FIGURE 15

Polygraph recordings of mechanical contractions; comparison of responses to semi-close arterially injected dTc (at the arrows) in the treated and control anterior tibialis muscles of rabbits (in situ), during indirect electrical stimulation. Supramaximal twitches were elicited once every 2.5 seconds.

A -- Normal (intact) control.
B -- "Blank"-implant control (implant in place for 14 days).
C -- Lidocaine-silicone polymer treated sciatic nerve, on the 14th day of continuous neuromuscular block [Group II(14d)].

The interruption in the records, within each frame, indicates a time lapse of about 6 minutes, at which time the amplitude of the twitch recovered to an approximately pre-injection level.
## TABLE 8
### SUMMARY OF PHARMACOLOGICAL FINDINGS

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>ACH Threshold Dose</th>
<th>ACH &quot;Massive-Response&quot; Dose</th>
<th>Responses to SCh and dTc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical Denervation (Group I)</td>
<td>0.1 ug</td>
<td>4.0 ug</td>
<td>Transmission ceased on or after the 3rd day following denervation.</td>
</tr>
<tr>
<td>Lidocaine Block 1-14 days (Groups II &amp; III)</td>
<td>50 ug</td>
<td>400 ug</td>
<td>Absence of denervation changes; responses identical with those in control muscles.</td>
</tr>
<tr>
<td>Controls -- &quot;Blank&quot;-Implant &amp; Intact (Groups IV &amp; V)</td>
<td>50 ug</td>
<td>400 ug</td>
<td>Neuromuscular transmission normal; SCh and dTc cause effective neuromuscular blockades</td>
</tr>
</tbody>
</table>
HISTOPATHOLOGICAL OBSERVATIONS

Gross observation of the sciatic nerve of the lidocaine implant treated animals showed no swelling or discoloration of the segment which had been surrounded by the implant.

Twelve gross specimens of lidocaine treated sciatic nerve segments, together with four "blank"-implant treated segments and two intact sciatic nerve specimens were fixed in 10% neutralized formaldehyde solution (Zenker's Formalin).

Preliminary histological examination of the specimens failed to reveal any significant light microscopic changes in the treated nerve segments as compared with segments of normal sciatic nerves.
CHAPTER V
DISCUSSION

THE NEW METHOD OF MUSCLE INACTIVATION

Research approaches and methods, aimed at the elucidation of the influence of motor innervation on the skeletal muscle, have been varied and numerous. One of the most important and highly controversial aspects of the problem is whether the transmission of nerve impulses is identical with, or at least plays a significant role in, the neurotrophic function. In order to examine this point, the method employed for the elimination of nerve impulses is crucial, and should be exclusive and without the introduction of variables or experimental artefacts.

As indicated in the statement of the problem and in the introduction, various methods employed to examine this problem, prior to the present study, failed to fulfill the requirements for an adequate experiment. The new method used in this study proved to be a satisfactory experimental device and made this investigation possible; it will also be useful in further examination of related aspects of the problem. For this reason, inclusion of an evaluation of the method in the discussion seems to be in order.

The method used in this study, to our knowledge, is entirely new. This unique approach is non-destructive and reversible, and it introduces a minimum of variables. In the muscle-inactivation preparations in this dissertation, no destructive or irreversible procedure was employed, and there was no interference with input nerve impulse traffic to the motoneuron
(i.e. there was no cordotomy or rhizotomy), except for interference with feedback through the blocked nerve. This is a much simpler and more direct method of muscle inactivation, without motor denervation, than methods reported previously (Tower, 1939b; Tower et al., 1941; Eccles, 1941b; Denny-Brown and Brenner, 1944; Johns and Thesleff, 1961). It is, for example, devoid of some of the destructive, although reversible, features of the nerve-compression method used by Denny-Brown and Brenner (1944). The lidocaine-implant method is far more reproducible and controllable.

The method of muscle inactivation by local anesthetic injected perimuscularly, or the alternate method of sciatic nerve block, used by Sokoll et al. (1968), cannot be considered as a "simple" prolonged neuromuscular impulse deprivation. First, any possible toxic and/or metabolic effects of the drug bathing the muscle were not ruled out; then, the method, as used, was reported to produce only an intermittent blockade of the sciatic nerve, for 7 days; and, further, the completeness and continuity of the electrical silence in the muscle was not examined.

The method used by Gutmann and Zak (1961), using local anesthetic for a reversible conduction block, is similar, in concept, to the method used in the present study. The 3 days of duration, however, is quite short. The aim of that study was to assess biochemical changes, and the electrophysiological and pharmacological effects of muscle inactivation were not assessed.

Careful review of the literature has failed to reveal any study, using any method whatsoever, that included a comprehensive evaluation of the
effect(s) of a prolonged simple neuromuscular impulse elimination in regard to the classical triad of signs (elevated chronaxie, fibrillation potentials, and acetylcholine supersensitivity) of denervation. The method used in the present study appears to have made available a technique for the study of such findings.

THE MODE OF ACTION OF LOCAL ANESTHETICS

The mode of action of local anesthetics is not fully known. The ability of these agents to block conduction of nerve impulses, in a reversible manner, has been attributed to several mechanisms of action. Harvey (1939b) proposed that procaine had a curare-like action in that it produced a depressant effect on the motor end-plate; in addition, Harvey (1939b) reported, procaine also had a depressant action on the motor nerve endings and on preganglionic nerve endings. He demonstrated that procaine interfered with the normal peripheral output of ACh during preganglionic stimulation.

Higman and Bartels (1961) reported that local anesthetics compete with ACh for receptors, by inhibiting the ACh-receptor protein, and block the membrane without depolarization, similarly to the action of curare. Condouris (1963) showed that a competitive antagonism exists between extra-cellular sodium ions and procaine. Similarly, Inoue and Frank (1962) demonstrated that procaine suppressed the specific increase in sodium conductance which normally follows an adequate stimulus. Shanes (1963), in his review, considered the various electrophysiological theories to be more acceptable than the receptor theory of Higman and Bartels (1961).
On the basis of a recent review on the mode of action of local anesthetics, by Ritchie and Greengard (1966), it seems that more creditable views on the mechanism of action of local anesthetics begin to be available. Evidence is increasing that local anesthetics block conduction by affecting the generation of nerve action potentials, i.e. by interfering with sodium and potassium conductance changes. Local anesthetics act on or at the nerve membrane and are active in their cationic form. They appear to affect the physico-chemical state of the membrane constituents which subsequently leads to changes in the ionic permeability of the membrane. These permeability alterations ultimately result in the conduction block (Ritchie and Greengard, 1966).

THE FINDINGS ESTABLISHED BY THIS INVESTIGATION

Comparison of the data, presented in Results, indicate that the effects of the prolonged (up to 14 days) neuromuscular impulse deprivation on the anterior tibialis muscle of the rabbit show no similarities to the electrophysiological and pharmacological changes which occurred in the surgically denervated muscle. In fact, the electromyographic, electrodiagnostic and pharmacological test reactions of the lidocaine inactivated muscles show a remarkable resemblance to the test reactions of the normal muscle, indicating the absence of the classical triad of signs of denervation supersensitivity.

Absence of Electromyographic Changes

The onset of nerve conduction block of the sciatic nerve was represented on the EMG by the disappearance of motor unit potentials, leading to
electrical silence, i.e. to an essentially straight baseline. This silence remained unchanged during the various periods of neuromuscular impulse deprivation in the anterior tibialis muscles (Figure 2, A,B,C). The baseline was essentially the same as the one in the normal muscle at complete rest (Figure 4, A), or the electrical silence in the surgically denervated muscle immediately after nerve section (Figure 1, A). In contrast to the normal muscle, however, no motor units could be produced in the lidocaine blocked muscles by passive movements of the limb, and no muscle action potentials could be elicited by attempting reflex responses with nociceptive stimuli (Figure 2, A). This indicated that the degree of muscle inactivation was equal to that in the freshly denervated muscle (Figure 1, A) which was completely deprived of neuromuscular impulse activity by means of motor nerve section. In contrast to the surgically denervated muscles, however, in the lidocaine inactivated muscle the electrical silence continued and was not replaced by spontaneous electrical activity, i.e. by fibrillation potentials, or any other EMG potentials, during the entire period of inactivation.

In regard to the electromyographic signs of denervation supersensitivity, the above findings clearly indicate that the effects of a prolonged neuromuscular impulse elimination (up to 14 days), in the anterior tibialis muscle of the rabbit, show no resemblance to the EMG changes which follow surgical denervation (Denny-Brown and Pennybacker, 1938; Tower, 1939b) or the various other procedures, such as botulinum treatment (Thesleff, 1960a), tetanus treatment (Prabhu and Oester, 1962), or experimental vitamin-E deficiency (Fudema et al., 1960a).
EMG Following Reversal of the Block

When the neuromuscular impulse deprivation was reversed, by removal of the lidocaine-silicone polymer implant or allowing its depletion of the drug reservoir in situ, the electrical activity gradually re-appeared in the previously inactivated muscles. This occurred not in the form of any spontaneous fibrillation potentials or of other abnormal EMG potentials, but began with a few normal type motor units, with characteristic EMG parameters of the normal muscle (di- and triphasic; 100 to 2000 microvolts; 2 to 10 milliseconds). These motor unit potentials were produced by passive movements of the previously paralyzed limb, in a few hours after removal of the nerve-conduction block (Figure 3, A). When this muscle was made to contract forcefully, e.g. by evoking a strong withdrawal response with nociceptive stimulation, a full interference pattern appeared on the oscilloscope (Figure 3, B). When the animal was allowed to relax, EMG in the resting muscle showed essentially electrical silence (Figure 3, C).

These EMG recordings from the muscles, which readily recovered from the inactivation, were essentially the same as those obtained in intact, normal anterior tibialis muscles of the rabbit. In a normal muscle, electromyography reveals a few simple electrical potentials, i.e. the individual motor units upon minimal contraction. When the muscle is made to contract stronger, the number of discharges, i.e. the number of individual motor unit potentials, increases, and more and more additional motor units are brought into action. On moderate contraction, this may result only in a "partial interference pattern". A strong and forceful
contraction of a normal muscle will, in any case, result in a full "interference pattern", due to the summation of a maximal number of motor unit potentials, in which the individual motor units cannot be recognized because the baseline is practically obliterated (Figure 4, C). Such interference pattern is not attainable in a denervated muscle. More important is the fact, however, that in a normal muscle, when it is allowed to relax completely, an essentially isoelectric baseline (electrical silence) can again be obtained (Figure 4, A). This was the case in the lidocaine inactivated muscles following reversal of the block (Figure 3, C); in contrast, however, such electrical silence is not recorded from a chronically denervated muscle.

The normal EMG findings in the inactivated muscle, during the nerve conduction block and after reversal of the block, exclude the possibility that the prolonged neuromuscular impulse deprivation had some untoward effects on the muscle, detectable by EMG. In addition, repetitive nerve stimulation in the re-activated (previously lidocaine blocked) muscles showed no decrease or increase in the amplitude of muscle potentials or in the parameters of stimulation. A denervation process would have initiated some fibrillation activity; the first signs of such spontaneous electrical activity generally appear in about 29-36 hours after nerve section and are characterized by single potentials of low amplitude (about 30 microvolts; 2-3 milliseconds duration), as reported by Gutmann et al. (1955) and Luco and Eyzaguirre (1955). If re-innervation fails to take place, fibrillation potentials may persist for months or even years, and may be
present as long as the individual muscle fibers remain denervated and are still capable of contracting (Tower, 1939b). The findings after reversal of the block exclude, or at least diminish, the possibility of a re-innervation process -- had there been some focal denervation changes that were unnoticed. In the course of re-innervation there is approximately a 2 weeks interval between the disappearance of fibrillation activity and the re-appearance of EMG potentials on attempting contraction (Feinstein et al., 1945). With the frequency of EMG examinations and the multiple electrode insertions used in this study, the likelihood, that isolated or temporary changes were unnoticed by EMG, is quite small.

Fibrillation and Theories of Its Mechanism

In the surgically denervated muscle, the initial electrical silence remained for only 2 or 3 days after nerve section (Figure 1, A). During this period, passive movements of the paralyzed limb or nociceptive stimulation of the animal failed to elicit any EMG potentials in the denervated muscle. From the 3rd day on, a moderate "insertion activity" ("irritation potentials"), occasional positive sharp waves, and few or several spontaneous fibrillation potentials were recorded (Figure 1, B). The latter gradually increased in number, giving a widespread fibrillation that persisted throughout the observations (Figure 1, C). These spontaneous fibrillation potentials are known to remain during ether or sodium pentobarbital anesthesia (Feinstein et al., 1945); in the present study, ether anesthesia was used to confirm the persistence of fibrillation in denervated muscles. In an occasional lidocaine preparation, when complete rest of the animal could not be achieved, the absence of fibrillation was confirmed under ether anesthesia.
Spontaneous fibrillation is an inevitable electrical manifestation of denervation in skeletal muscle. Despite the number and variety of studies attempting to elucidate its cause(s), the mechanism of action by which these potentials are generated is still not clear. It is known that fibrillation occurs following axonal section anywhere along the peripheral motor neuron, from anterior horn cell to the terminal arborization at the end-plate. It does not occur in supra-horn cell lesions, or in muscle atrophies following tenotomy or immobilization (Tower et al., 1941; Solandt et al., 1943; Johns and Thesleff, 1961). Although Langley and Kato (1915a) thought that fibrillation is due to contraction of only a part of the muscle fiber, Eccles (1941a) demonstrated that the entire muscle fiber contracts. Hayes and Woolsey (1942) and Jarcho et al. (1954) showed that the impulse leading to this contraction originates in the end-plate region.

Several postulates in the past suggested ACh as the agent responsible for fibrillation in a denervated muscle (Denny-Brown and Pennybacker, 1938; Bergamini et al., 1955). The observation that curare does not depress fibrillation, serves as strong evidence against such a postulate (Rosenblueth and Luco, 1937; Eccles, 1941a). The lack of direct and exclusive causal relation between ACh and fibrillation has been further demonstrated by other reports (Mead, 1947; Bowman and Raper, 1964). It has also been reported, for example, that an electrical stimulus can elicit repetitive discharges in a denervated muscle, comparable to spontaneous fibrillation potentials (Eccles, 1941a). Li et al. (1957) demonstrated
that fibrillation may be produced by an autogenous (self-regenerative) rhythmic oscillation of the membrane potential, and Li (1960) reported, further, that generation of these potentials does not have the requisite of a preceding degeneration of the end-plate. Injection of potassium chloride, intravenously, intensifies fibrillation (Magladery and Solandt, 1942), but this may be due to the already existing alteration of membrane potentials (Eccles, 1963).

Attempts to correlate fibrillation with other changes of denervation supersensitivity have been inconclusive. For example, the hypothesis that persistent fibrillation is the cause of muscle atrophy (Langley and Kato, 1915b; Tower, 1939b) proved unsatisfactory because, when fibrillation was suppressed by quinidine, the muscle atrophy continued to progress (Solandt and Magladery, 1940), and, in some cases, muscle atrophy was reported to occur earlier than fibrillation (Feinstein et al., 1945). Josefsson and Thesleff (1961) attributed fibrillation to the disappearance of m.e.p.p.'s; and the already unlikely causal relationship between ACh and fibrillation became even less clear by the finding that curare failed to prevent or diminish the extension of ACh-receptor surface in the surgically denervated muscle (Axelsson and Thesleff, 1959). It is also known that in certain clinical muscle atrophies in which fibrillation is absent, some degree of ACh sensitivity was reported; for instance, after immobilization by de-afferentation and cordotomy (Solandt and Magladery, 1940), and after immobilization by skeletal fixation (Solandt et al., 1943). Desmedt (1950) indicated some correlation between electrophysiological changes and
fibrillation, reporting that chronaxie reached a maximal value at the
time when fibrillation activity was at its height. There is no doubt that
a satisfactory explanation for the mechanism of fibrillation, and a better
correlation between signs of denervation sensitization would contribute
to the elucidation of the neurotrophic concept.

Absence of Electrodiagnostic Changes

Comparison of EDX data indicates that the parameters of electrical
excitability of the inactivated muscles remained unaltered during the
prolonged neuromuscular impulse deprivation. As graphically presented
in Figures 5-9, no differences can be seen between the slope and position
of the S-D curves, in the continuity and flatness of repetitive stimulation
curves, or in the chronaxie and rheobase values, obtained in the lidocaine
block inactivated muscles, as compared to corresponding data in normal
controls.

Normal rheobase and chronaxie values do not necessarily supply
reliable information that the muscles tested are still intact in regard
to motor innervation. Rheobase, alone, is considered to have a question-
able diagnostic value (Oester and Licht, 1956; Ritchie, 1954). It has
been reported to remain unaltered during short-term denervation and to
remain relatively unchanged in prolonged partial denervation (Wynn-Parry,
1953). A normal chronaxie may be obtained in the presence of denervation
changes of a focal or diffuse distribution in the muscle. Therefore,
a normal chronaxie is not a safe index that the muscle is not partially
denervated (Grundfest et al., 1956). In order to rule out any denervation
changes, one should not rely on these measurements alone (Harris, 1956). On the other hand, chronaxie values can be closely correlated with the regeneration stages of the neuromuscular complex (Grundfest et al., 1956). Pollack et al. (1945) and Woodhall et al. (1956) still consider chronaxie to be a useful method for orientation about the direct excitability state of the muscle.

A full strength-duration curve is a very reliable representation of excitability characteristics of the tissue being stimulated (Wynn-Parry, 1956). It is true that it gives information only about the superficial muscle fibers; its application, however, especially in conjunction with electromyography, offers a valuable picture of the innervation state of the muscle, including the degree of partial innervation in the course of regeneration. It has a particular value in that it may give evidence of denervation, following nerve injury, earlier than electromyography, according to Lambert (1960). Richardson (1951) reported that S-D curves revealed the denervation, in chronic motor neuron disease, prior to detection of fibrillation by EMG.

Electrodiagnosis of muscle with repetitive stimulation was reported to give a characteristic measure of the state of innervation (Pollack et al., 1947). In a denervated muscle, the characteristic increase in threshold amperages, at all conventionally used frequencies, became manifest. In contrast, the normally innervated muscle had about the same current requirements for tetanic contraction at all frequencies.

With the rigorous and constant techniques used in this investigation, these EDX methods are regarded as useful diagnostic studies for the
detection of denervation changes. The uniformity of the negative EDX findings in the inactivated muscles, in conjunction with the negative EMG and pharmacological findings, strongly suggests that the prolonged neuromuscular impulse elimination had no significant untoward effects on the inactivated muscles. Despite some of the inherent weaknesses of the individual EDX methods used, the combined application of these methods decreases the likelihood that the findings were "false-negative".

Absence of Pharmacological Changes

Sensitivity to ACh. Responses of the nerve impulse deprived muscles to semi-close arterially injected test doses of ACh were essentially identical with corresponding responses of the normal anterior tibialis. This finding was the same whether the inactivated muscles were tested while the neuromuscular impulse elimination was still uninterruptedly in effect, or after the nerve conduction block had been reversed.

The close similarity between the ACh-sensitivity of the inactivated and of the normal muscles was in sharp contrast with the unequivocal and marked changes in ACh sensitivity of chronically denervated muscles. In the latter, the threshold dose of ACh was about 500 times smaller than that in the lidocaine inactivated and in normal muscles (Figure 10, A). This sensitivity change corresponds to, and is within the range of the 100 to 100,000 fold change in ACh threshold which has been reported, dependent upon the species used and the method of ACh application (Brown, 1937; Rosenblueth and Luco, 1937; Reid and Vaughan Williams, 1949; Nicholls, 1956).
The marked and prolonged contracture and the diminished twitch response to electrical stimulation during the contracture (Figure 10, B), as a response to a "massive" dose, i.e. to the injection of a mere 4.0 ug of ACh, is a characteristic finding in denervated muscle and corresponds to observations by Dale and Gasser (1926) and Brown (1937). As shown by the recording (Figure 11), no such ACh sensitivity changes were found in the inactivated muscles.

Since the early reports (Frank et al., 1922; Brown, 1937; and others) that, within a few to several days after denervation, such increase in ACh sensitivity occurs in the muscle deprived of its motor nerve supply, this phenomenon has been examined from many and diverse points of view (Gutmann, ed., 1962; Gutmann and Hník, eds., 1963). The chemical supersensitivity, almost invariably, occurs together with the electrophysiological changes of denervation supersensitivity (Brown, 1937; Tower, 1939b; Desmedt, 1949). The parallel development of multiple supersensitivity signs has often been reported, also, after various procedures in which there was some interference with the physiological or metabolic integrity of the neuromuscular transmission mechanism (Fudema, 1958; Oester et al., 1959; Fudema et al., 1960a and 1960b; Thesleff, 1960a; Josefsson and Thesleff, 1961; Prabhu, 1962; Prabhu and Oester, 1962 and 1963; Prabhu et al., 1961 and 1962b).

It is generally believed (Desmedt, 1959; Gutmann, ed., 1962; Gutmann and Hník, eds., 1963; Sharpless, 1964) that inactivation of the skeletal muscle without motor nerve section does not result in ACh sensitivity changes, although rare cases of observed ACh sensitivity (of considerably
lesser magnitude than in denervation) have been reported in motor-innervated but immobilized muscles (Solandt and Magladery, 1940; Solandt et al., 1943). Muscle atrophy also occurred, concurrently, but fibrillation was absent.

The chemical supersensitivity is not due to changes in electrical properties of the muscle membrane (Nicholls, 1956); and the causal relationship between ACh-receptor changes and supersensitivity to ACh is not settled (Miledi, 1960a and 1962; Thesleff, 1960b). Since the report of Axelsson and Thesleff (1959) showing extension of ACh sensitivity over the entire sarcolemma in denervated muscles, no fully satisfactory explanation for this mechanism has been offered (Miledi, 1962 and 1963; Thesleff, 1960a and 1960b; Gutmann and Hnik, 1962). The question is wide open whether the junctional subthreshold quanta of ACh (Fatt and Katz, 1952) is the factor which is crucial in the maintenance of the trophic influence of the muscle and whether nerve impulses and/or the neurotransmitter complex are actually indispensable for, or may be dissociated from, the still unidentified regulatory mechanism (Thesleff, 1960b; Miledi, 1960a, 1960c, 1962, 1963; Gutmann, 1963).

It is strongly maintained, however, that the increased sensitivity to ACh and other pharmacological agents, and the characteristic electrophysiological and other changes (morphological, metabolic) result from a possible common cause: the alteration of the physiological-metabolic balance of the muscle (Desmedt, 1959; Eccles, 1963; Gutmann, 1963). For this reason, the postulates for the development of ACh supersensitivity will be treated in a later section, under a general discussion of the
neurotrophic concept, including some of the postulated and known effects of disuse on the muscle. An addition to the latter is the finding, established in this study, showing that a simple and reversible, prolonged neuromuscular elimination of nerve impulses, failed to initiate an increased sensitivity to ACh.

Responses to injected SCh and dTc. The absence of pharmacological supersensitivity in the inactivated muscles, during and after the prolonged neuromuscular impulse elimination, is further indicated by the responses to semi-close arterial injections of SCh and dTc, as compared to corresponding responses of the normal muscle. In the normally innervated skeletal muscle, these agents are known to cause either a depolarizing neuromuscular blockade (SCh) or a non-depolarizing blockade (dTc). Comparison of data, as presented in Results, (see Figures 14 and 15), shows essentially similar responses in the preparations compared. The drug induced neuromuscular blockade is approximately the same in both the inactivated muscles and controls.

In a denervated muscle, in about 29-36 hours after nerve section, an electrical stimulus applied to the distal nerve segment (the stump attached to the muscle) elicits no muscle contraction, and morphological changes affecting the nerve endings are noted (Gutmann and Holubar, 1950). Recently, Okamoto and Riker (1969) reported that the initial functional changes after denervation occur at the motor nerve terminals, and that characteristic transmission losses become manifest in about 48 hours after nerve section. In the denervated anterior tibialis muscles in this study, the absence of
muscle response to indirect electrical stimulation corresponds to the above findings. In contrast, at all times during the prolonged nerve conduction block or after recovery from this inactivation, the effectiveness of the indirect electrical stimulation in the lidocaine-block inactivated muscles revealed no evidence of transmission loss. Prior to injections of the neuromuscular blocking drug, the indirectly elicited muscle twitches were about the same in the inactivated muscles and controls (Figures 14 and 15).

The site(s) of action of SCh and dTc, i.e. whether these drugs act on pre- or postsynaptic sites, is still a point of controversy at present. Standaert (1964) reported that the principal site of action for dTc was at the motor nerve terminals, and Standaert and Adams (1965) reported that SCh primarily and profoundly affects the motor nerve terminals, in addition to, and independently of, its post-junctional action. Karczmar (1967) in his thorough review of neuromuscular pharmacology, and on the basis of an analytical review of the pertinent literature, considers the concept of postsynaptic mechanisms as having been satisfactorily explained and documented. It has also been postulated by Blaber and Karczmar (1967) that "multiple cholinoceptive and related" sites are present in the (cat's) neuromuscular junction.

The brief examination of the functional status of the neuromuscular junction in inactivated muscles, by the above method, does not add any information in regard to site(s) or mechanism of action of these drugs. The close resemblance of the neuromuscular blockades, in the inactivated
muscles, to a "normal" type action of the respective drugs, suggests that gross functional alterations are unlikely to be present at the neuromyal junctions after the prolonged inactivation. The absence of depolarization by dTc, for example, in the lidocaine preparations, further suggests the absence of significant denervation changes; dTc is known to depolarize (i.e. to produce contraction in) the denervated but not the normal (innervated) muscle (Bowman and Raper, 1964).

Absence of Palpable Muscle Atrophy

Skeletal muscle undergoes rapid atrophy after motor nerve section (Tower, 1935 and 1939a; Gutmann and Young, 1944; Sunderland and Ray, 1950; Hnik, 1962), but muscle atrophy of varying degree also occurs in immobilization of muscle without motor denervation (Tower, 1937; Eccles, 1944; Ferguson et al., 1957; and others). In the present study, the progressive palpable atrophy of the denervated muscles is consistent with the above reports.

In the inactivated muscle, without motor denervation, the mechanism of muscle atrophy is not known, and the role of activity/or muscle contraction, and especially of nerve impulses is highly controversial. Several investigators suggested that it is the absence of tension in the muscle, or the inability of the muscle to develop effective tension, that leads to atrophy (Solandt et al., 1943; Eccles, 1944; Ferguson et al., 1957). Eccles (1944) reported, for example, that the progression of atrophy could be slowed down by brief daily electrical stimulation in the immobilized
(de-afferented) muscle, but this had no effect in the tenotomized muscle because it could not develop effective tension. In contrast, McMinn and Vrbova (1967) reported that "activity" (isotonic contractions) was the cause of rapid degeneration in the tenotomized soleus of the rabbit. This rapid degeneration could be prevented by sectioning the motor nerve or by cordotomy; these authors offered no postulate for the mechanism.

Denny-Brown and Brenner (1944) suggested that atrophy can be prevented in the paralyzed muscle even in the absence of nerve impulses, provided that the anatomical integrity of the motor neuron is preserved. Integrity of the motor nerve is considered to be essential to prevent atrophy, but not through the ability of the nerve to conduct impulses; it is the "metabolic recovery process", in which both the muscle and the motor nerve participate, that has been regarded as the trophic mechanism (Gutmann, 1962; Gutmann and Hnik, 1962). For example, similar biochemical changes were reported in muscle atrophies due to denervation, tenotomy and vitamin-E deficiency, generally indicating an increased "metabolic turnover rate"; this may be a compensatory mechanism leading to atrophy (Ferdmann, 1963). A short-term inactivation of a muscle by nerve conduction block (Gutmann and Zák, 1961) reproduced none of the changes seen in denervated controls, except the "initial denervation hypertrophy", attributed by the authors to a possible slowing of catabolic processes. It is known, for example, that atrophy is more rapid in a muscle that was "exhausted" by activity before nerve section (Gutmann and Hnik, 1962).

In this investigation, quantitation of muscle mass (by volume, wet- or dry-weight, or by histology) was not done. However, the careful
frequent palpation of the inactivated limbs failed to detect any appreciable change in the muscle mass, using the contralateral muscle as reference. This finding indicates that prolonged absence of neuromuscular impulse activity did not cause any significant palpable muscle atrophy.

THE NEUROTROPHIC CONCEPT AND "DENERVATION SUPERSENSITIVITY"

The complexities of nerve-muscle relationship makes it difficult to escape the thought that a wide range of phenomena and functions may be regarded as "trophic". Evidence is growing, in fact, that only the nerve impulses and neuromuscular transmission may be excluded and everything else might be regarded as trophic (Denny-Brown and Brenner, 1944; Luco and Eyzaguirre, 1955; Desmedt, 1959; Eccles, 1963; Gutmann, 1963; Miledi, 1963; Sharpless, 1964).

The concept of "denervation supersensitivity", as postulated by Cannon and Rosenblueth (1949), implied that such changes are due to the cessation of some protective influence exerted in centrifugal direction, i.e. through the efferent pathways. Drake and Stavraky (1948) suggested the extension of the Law of Denervation to include (in the central nervous system) an afferent trophic mechanism; these authors reported that, following dorsal rhizotomy, ACh injection resulted in convulsive activity, presumably as a sign of supersensitivity of the de-afferented neurons. Eccles et al. (1962) reported, however, that the motoneuron itself is not affected by dorsal rhizotomy, and the hyperactivity was due to inter-neuronal imbalance; this can be regarded as a "release" phenomenon.
In the skeletal muscle, the trophic influence operates via the motor innervation, and no convincing evidence is available indicating that sensory or sympathetic fibers are involved (Gutmann and Hnik, 1962).

The essential, although yet unexplained, role of the motor nerve cannot be doubted; that this "trophic" role is separate from the conduction of nerve impulses, seems to gain more and more support. Some of these are derived from embryological studies (Orbeli, 1945; Diamond and Miledi, 1962). It is also likely that the trophic influence is conveyed along the proximo-distal flow of the axoplasm (Weiss and Hiscoe, 1948). For example, both ACh sensitivity and fibrillation occur earlier when section of the nerve is closer to the muscle (Luco and Eyzaguirre, 1955). Degeneration of the motor end-plate occurs later with a longer peripheral nerve stump (Gutmann et al., 1955). It is unlikely that nerve impulses or junctional transmission, per se, are trophic, because these cease immediately upon nerve section, regardless at what point the section is done. The axoplasmic transport is considered to be involved in the trophic mechanism (Gutmann et al., 1955; Luco and Eyzaguirre, 1955; Gutmann and Hnik, 1962; Gutmann, 1963); in a denervated muscle, it is the degeneration of nerve-muscle connections but not necessarily the cessation of nerve impulses that initiates the denervation changes (Gutmann and Holubar, 1950; Gutmann et al., 1955; Miledi and Slater, 1968).

A possible role of ACh in the trophic influence, which may not be connected with its impulse function, is highly controversial (Miledi, 1962 and 1963; Thesleff, 1960b). Thesleff (1960b) attributes a crucial, perhaps
exclusive, role to the spontaneous junctional quanta of ACh, pointing out that the same factor was responsible for the parallel appearance of ACh sensitivity and fibrillation after botulinum intoxication (Thesleff, 1960a; Josefsson and Thesleff, 1961) and after denervation. This factor is the great reduction in m.e.p.p.'s, that was reported to occur at the denervated neuromyal junction (Birks et al., 1960). Thesleff suggested that it was the junctional leakage of ACh that prevented the expansion of the ACh-sensitive area from end-plate to muscle membrane in an immobilized muscle (Johns and Thesleff, 1961), and the absence or decrease of this ACh leakage caused the sensitization of the entire membrane, as for example in the denervated muscle (Axelsson and Thesleff, 1959). In the botulinum intoxicated muscle, the frequency of m.e.p.p.'s was inversely proportional to the area of ACh sensitive surface, and Thesleff considers the m.e.p.p.'s as being the "receptor controlling factor" (Thesleff, 1960a).

Miledi (1960b and 1962) strongly argues against ACh as the receptor controlling factor. He could not find any correlation between frequency of m.e.p.p.'s and development of ACh sensitivity. For example, the generalized chemosensitivity of the denervated frog muscle diminished as the regenerating axon established synaptic contact with the muscle, without significant increase in m.e.p.p. frequency. Further, ACh sensitivity became restricted to the end-plate prior to the capability of neuromuscular transmission (Miledi, 1960c).

It has also been reported that injury to the muscle itself can result in supersensitivity, in both the innervated and in the aneural portion of a
divided muscle (Katz and Miledi, 1961). Spontaneous ACh sensitization also exists in the foetal muscle, prior to the development of innervation (Diamond and Miledi, 1962). The increased reactivity in the denervated muscle is likened to the characteristics of the embryonic muscle at the early stages of ontogenetic development (Orbeli, 1945; Gutmann, 1964). This is thought to be an inherent, autogenous characteristic of the muscle cell itself; it becomes depressed or modified by the trophic neural influence of motor innervation, but not by the conduction of nerve impulses (Gutmann, 1962). In denervation, the muscle may revert back to its embryological stage (Gutmann and Hník, 1962); but the trophic control may be rendered ineffective also by other means. For example, by injury to the muscle (Katz and Miledi, 1961) or by a variety of causes which will lead to changes in the muscle's metabolic balance (Bass, 1962), but which are not caused by the cessation of nerve impulses (Gutmann, 1959, 1962, 1963; Gutmann and Hník, 1962; Miledi, 1963).

Eccles (1963) considers that it is the "slowing of the contractile process" in the denervated muscle which directly leads to the biochemical changes. Nerve cross-union experiments (Buller et al., 1960), for example, showed that the "contractile speed" of the muscle is controlled by the motor nerve and that it can be altered (i.e. a "fast" muscle becomes "slow" and vice versa), by nerve cross-union. Similarly, the slowing of the contractile process in the denervated muscle is reversed when the nerve regenerates. This reversal of slowing occurs prior to nerve impulse conduction, and in spite of the absence of nerve impulses (Eccles, 1941b and
1963; Gutmann, 1963). Furthermore, this slowing of contractile process does not occur in simple disuse (Gutmann and Hnik, eds., 1963).

It is apparent that the term "denervation supersensitivity" is becoming less and less definitive and may even be inappropriate; its use may be justified only as an analogy, or perhaps when employed in an historic sense. The wide range of alterations encompassed by the term "denervation supersensitivity" are not restricted to a muscle that is denervated but analogous changes develop, in an innervated muscle, from various other causes (physiological, metabolic, or bio-molecular), leading to similar dysfunctions (Fudema et al., 1960a and 1960b; Gutmann and Žák, 1961; Katz and Miledi, 1961; Gutmann and Hnik, 1962; Bass, 1962; Prabhu et al., 1962b; Žák, 1962; and others). On the same grounds, "pharmacological denervation", by Emmelin (1961), also seems inappropriate.

In place of the extension of Cannon's Law of Denervation, a re-definition of the phenomenon seems more justified.

COMPARISON WITH OTHER INVESTIGATIONS

It is difficult to make a point to point comparison between the results of this investigation and the findings of earlier studies. The reason is that both the method of muscle inactivation and the duration of neuromuscular impulse elimination were so different.

The electrophysiological and pharmacological findings in denervated muscles, which served as controls, are in general agreement with those of earlier investigators.
The "negative" findings in the lidocaine-block inactivated muscles, on the other hand, established new data which serve as experimental support for the postulates of other workers who felt that the conduction of nerve impulses is not identical with, and may be separated from, the neurotrophic mechanism. The lidocaine-block technique successfully separated nerve-impulse conduction from the trophic functions of nerve.

This study produced no experimental data that would directly contribute to the controversial role of m.e.p.p.'s or of ACh in the neurotrophic relations.

It is sincerely felt, however, that the findings established in this study do contribute to the elucidation of the problem. As a note of caution, it may be possible that some changes, not discernible, and detectable only with other techniques or different methods, occurred in the inactivated muscles. Or, it is conceivable, that a nerve-impulse deprivation lasting much longer than 14 days, would allow some alterations to become manifest; this is based on a possible assumption that the consequences of denervation and muscle inactivation might be analogous but their dynamics operate on different time scales.

FUTURE PROBLEMS

Several additional aspects of the problem need to be studied. For example, the neuromyial junction: ACh release during inactivation and following stimulation of the nerve distal to the block, with varying frequencies, for various durations, and after varying intervals of nerve-impulse deprivation. This may supply useful data in regard to the dynamics
of neurotransmitter storage, release, re-synthesis and transport, during prolonged complete inactivation.

Histological and electron-microscopic studies of the junctional structures and the muscle, after various periods of inactivation, would be useful.

Repeat of the studies of the present investigation, both, longer in duration and in different species of animals, is indicated.

On a broader scale, the present model may be useful in biochemical studies of nerve and muscle metabolism.

CONCLUSIONS

In general, the conclusions based on the findings of this study, do not support some current concepts; namely, that prolonged absence of neuromuscular impulse activity in skeletal muscle, even in the absence of motor nerve section, would induce supersensitivity changes.

The prolonged and continuous elimination of neuromuscular impulses in the anterior tibialis muscles of the rabbit, by means of a lidocaine-silicone polymer implant around the sciatic nerve, failed to produce signs of denervation supersensitivity. In a direct comparison with the findings in the surgically denervated anterior tibialis muscle, no similarities were found which would point to the initiation of a denervation process as a consequence of the continuous pharmacological blockade of the motor
nerve. Although the findings of this investigation do not include any positive data that would indicate the nature of the trophic neural influence, this study has added to the elucidation of the role of nerve impulse traffic in relation to skeletal muscle.

The absence of electrophysiological and pharmacological alterations characteristic of denervation supersensitivity, both during the prolonged neuromuscular impulse deprivation of the muscle and after the reversal of that inactivation, indicate that the trophic neural influence may be independent of the concomitant presence of nerve impulses.

It is conceivable, of course, that the period of pharmacological nerve conduction blockade was, perhaps, too short to allow the operating trophic mechanism(s) to undergo alterations sufficient for the supersensitivity changes to become manifest. Without further experimental data, it is not possible to predict whether the reversibly inactivated muscle would undergo any of the "denervation supersensitivity" changes during a period considerably longer than the one used in this study. The longest duration of neuromuscular impulse elimination in this work, however, was about seven-fold of the time-course within which the surgically denervated muscles developed significant electrophysiological and pharmacological alterations.

Presumably, associated with the prolonged nerve conduction block, there was a corresponding reduction in chemical transmitter activity at the neuromyal junction. This, in turn, is presumed to have changed the balance of synthesis, storage and availability of the biochemical components of the cholinergic transmitter mechanism.
By exclusion, the findings presented in this study, point to the necessity of some anatomical, pathological, or bio-molecular change or process, or a combination of these, other than absence of nerve-impulse traffic, as prerequisite to the electrophysiological and pharmacological changes routinely obtained in the denervated muscle.

The hypothesis is offered that the conduction of nerve impulses is not essential in the trophic function of the motor nerve on the muscle.
CHAPTER VI
SUMMARY

The anterior tibialis muscle of the rabbit was subjected to a continuous deprivation of neuromuscular impulses, for up to 14 days. This was accomplished by an uninterrupted pharmacological nerve conduction block of the sciatic nerve by means of a lidocaine-silicone polymer implant, in situ. This prolonged muscle inactivation was non-destructive and readily reversible.

The effects of the non-destructive, prolonged, and reversible muscle inactivation were compared to the sequelae of surgical denervation and with corresponding electrophysiological and pharmacological parameters of normal muscles. The findings are summarized in the following categories:

CLINICAL NEUROLOGICAL FINDINGS

1) Chronic surgical denervation (sciatic nerve) resulted in an immediate complete flaccid paralysis of the limb, followed by the occurrence of palpable progressive atrophy of the anterior tibialis muscle. This paralysis was not reversible.

2) Lidocaine nerve conduction block also resulted in an immediate complete flaccid paralysis of the limb, equal to that in the surgically denervated control. In contrast, however, no palpable atrophy of the muscle was observed at any time, and this paralysis was readily reversible.
ELECTROMYOGRAPHIC FINDINGS

1) Both the surgical nerve section and the lidocaine nerve-conduction block, resulted in immediate disappearance of all motor unit potentials in the corresponding anterior tibialis muscles.

2) In the surgically denervated muscle, the electrical silence was replaced (from the 3rd or 4th postoperative day) by spontaneous and progressively increasing fibrillation-type potentials, persisting throughout the observations.

3) In contrast, in the lidocaine blocked muscles, the electrical silence remained unchanged throughout the periods of observation, up to 14 days, without the occurrence of spontaneous fibrillation potentials, or any other abnormal EMG potentials, at any time.

4) Removal of the lidocaine blocking implants resulted in rapid return of normal EMG findings, identical to those in intact, untreated control muscles.

ELECTRODIAGNOSTIC FINDINGS

1) Following surgical nerve section, the rheobase of the involved muscle, (after a brief initial elevation) fell markedly below the normal threshold. The chronaxie became elevated to 75-fold of the normal, by the 14th postoperative day. The strength-duration curve showed a marked increase in slope and alteration in position. The repetitive stimulation indices showed a marked increase in threshold and a change in pattern with different frequencies.
2) In contrast, the lidocaine blocked muscles showed no change in rheobase, chronaxie, strength-duration curve, and repetitive stimulation indices; these EDX parameters were essentially identical with those in normal muscles.

PHARMACOLOGICAL FINDINGS

1) Chronic surgical denervation resulted in a marked increase in sensitivity to close-arterially injected ACh, in situ. There was a 500-fold decrease in the threshold dose; the "maximal" response dose (eliciting a marked and sustained contracture) was less than one-tenth of the average threshold dose in normal muscles, and one-hundredth of the average normal "maximal" response dose.

2) In contrast, the lidocaine blocked muscles showed no change in ACh sensitivity at any time during the periods of neuromuscular impulse deprivation (up to 14 days), or after reversal of the block. Both the average threshold dose and the "maximal" response doses were essentially identical with corresponding doses in normal controls.

3) Responses to close-arterial injections of SCh and dTc, in situ, during indirect electrical stimulation, were essentially identical in both the lidocaine blocked and the normal control muscles, qualitatively and quantitatively. This finding indicates the absence of gross functional alterations in the responses of the neuromyot junction to these drugs during the prolonged neuromuscular impulse deprivation, up to 14 days, or after reversal of the muscle inactivation.
4) In contrast, in the surgically denervated muscles, indirect electrical stimulation was ineffective in eliciting muscle twitches, from the 3rd day on after nerve section. This indicated the absence of neuromuscular transmission secondary to denervation changes.

In general, the electrophysiological and pharmacological test reactions of the lidocaine-block inactivated muscles were essentially indistinguishable from those of the normal muscle, and were without any similarities to the test reactions of the denervated muscle.

The findings are discussed in the light of current concepts concerning the influence of motor innervation on the skeletal muscle and in the light of the new information made available from this study.

The data presented suggest that the trophic influence of nerve on skeletal muscle may be mediated independently of the continuous presence of nerve impulses and/or of neuromuscular transmission.
CHAPTER VII
APPENDIX

PREPARATION OF THE LIDOCAINE-SILICONE POLYMER IMPLANT (AND OF THE "BLANK"-IMPLANT)

The local anesthetic agent used for the prolonged axonal conduction block was incorporated into the polymer vehicle in the following manner:

A silicone polymer, still in the monomer form, SILASTIC-RTV® (Dow Corning Center for Aid to Medical Research, Midland, Michigan), a "room-temperature setting" polysiloxane, was thoroughly mixed with 20% lidocaine-base (XYLOCAINE®, Astra Pharmaceutical Products, Inc., Worcester, Massachusetts). The dough-like mass was pressed into specially designed molds and allowed to polymerize ("self-catalyzing") at ambient temperature. The resultant resilient small tubules were about 12 mm long, had an approximately 2 mm thick wall, weighing about 5 g, with 20% lidocaine-base content. The bore diameter was individually adjusted according to the thickness of the nerve trunk. The wall of the tubule was longitudinally slit for placement around the sciatic trunk during the implantation procedure. The implants were sterilized and preserved in sterile containers. At the time of surgical implantation, the lumen of the particular implant was carefully widened, if needed, for the desired loose fit around the nerve segment.

The implant served as a "slow-release" drug reservoir. In addition, the polymer, per se, has previously been shown to cause little or no foreign body reactions when placed in body cavities, around tissues or in body fluids, in situ (Brown et al., 1960).
The particular combination of drug/polymer used in this study, appeared to be void of any untoward properties, such as tissue damage in or around the enclosed nerve.

The actual mechanism of the "slow-release" properties of the drug/polymer combination in body fluids is not fully explained.

The "BLANK"-IMPLANT was prepared in a manner similar to that described above but without the addition of lidocaine-base.

SURGICAL TECHNIQUE FOR THE PLACEMENT OF THE IMPLANTS

Before surgery, the thigh of the animal was depilated in the operative area. The animal was then anesthetized by sodium pentobarbital (NEMBUTAL), 22 mg/kg, slowly injected into the marginal vein of the ear, while the animal was restrained in a specially designed box. As an anesthetic adjunct, 5 mg/kg atropine sulfate, i.m., was given.

After positioning and restraining the anesthetized animal on the operating table, the site was aseptically cleansed, the integument and subcutaneous fascia were longitudinally incised and the lateral intermuscular septum in the thigh was separated by careful blunt dissection to expose the sciatic trunk. At the midpoint of the thigh and proximal to the bifurcation of the common peroneal nerve, the implant was carefully slipped around the nerve. If the lumen of the tubule was too tight or too loose, the implant was removed and was adjusted for proper fit. In order to prevent trauma to the nerve by slipping or torsion of the implant while in situ, the tubule was secured to the surrounding fascia by means of a thin "sling" made of SILASTIC®-SHEATH, using 4-0 silk suture.
The site was kept moist with 0.9% saline and kept at 37°C by an infrared lamp. After 20-35 minutes, the conduction block was complete as assessed by the absence of muscle action potentials distal to the implant, while applying supramaximal single stimuli (square wave pulses of 0.1 msec duration, once every 2.5 seconds) by a Grass Stimulator (#S4G), to the sciatic nerve proximal to the implant. Total absence of electrical activity in the anterior tibialis muscle, during nerve stimulation, was monitored by intramuscular bipolar electrodes with an electromyograph (TECA Corp.).

The structures and tissues of the region then were properly repositioned and the site was closed. Skin closure was done by 2-0 silk; the closure was finished by spraying the area with antiseptic polyvinyl-pyrrolidone solution.

For prophylaxis, intramuscular tetracycline-HCl (ACHROMYCIN, Lederle Laboratories), 10 mg/kg, was given in the opposite (not-operated) thigh muscles or into the contralateral gluteus muscles.

Postoperatively, periodic inspection of the wounds was carried out in order to minimize artifacts from inflammatory or other untoward changes and to insure proper comfort of the animal.

The pre- and postoperative preparations, and the surgical procedures, including anesthesia, were identical for all treated animals.
CARE OF EXPERIMENTAL ANIMALS

The experiments throughout the studies reported herein were conducted according to the Guide for Laboratory Animal Facilities and Care (1965) prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences -- National Research Council; and according to the Laboratory Animal Welfare Act (Public Law 89-544, 1967).

DRUGS EMPLOYED IN THIS STUDY

ACETYLCHOLINE CHLORIDE -- (Merck)
ATROPINE SULFATE (Injections) -- (Burroughs-Wellcome)
SUCCINYLCHOLINE CHLORIDE -- (Squibb and Company)
d-TUBOCURARINE CHLORIDE -- (Abbott Laboratories)®
LIDOCAINE-BASE (XYLOCAINE-BASE) -- (Astra Pharmaceutical Products)®
SODIUM PENTOBARBITAL Injection (NEMBUTAL) -- (Abbott Laboratories)®
URETHANE
DIETHYL ETHER (Anesthetic Grade)®
TETRACYCLINE-HCl, Intramuscular (ACHROMYCIN) -- (Lederle Laboratories)®

All drugs, where dilutions or suspensions were needed, were freshly prepared with sterile water or sterile saline. Non-diluted preparations, after opening a container, were kept at temperatures and for the optimum length of time, as recommended by the manufacturer.
CHAPTER VIII

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APPROVAL SHEET

This dissertation submitted by Emery D. Robert 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.

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

25 May 70

[Signature]

Signature of Advisor