SOME EFFECTS OF PERIPHERAL NERVE CRUSHING ON THE SPASTICITY SECONDARY TO HEMISECTION OF THE SPINAL CORD

by

Leo Joe McMahon, B.A.

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INTRODUCTORY STATEMENT

A brief statement of the problem dealt with in this paper will be made at this time to provide orientation for the review of literature which follows.

There is evidence that the mammalian central nervous system possesses capacity to undergo morphological and functional alteration and reorganization under certain circumstances. In particular reference to this paper there is evidence to suggest that following injury to the spinal cord remaining intact axons are stimulated to produce collateral sprouting onto adjacent partially or totally denervated neurones, possibly as part of a compensatory mechanism.

Further there is evidence that this sprouting of collateral fibers is influenced by a variety of factors. Among these are use of the fiber, and injury to the parent cell. Previous work suggests that use of the intact collateral fiber stimulates sprouting of its peripheral processes if the proper stimulus to sprout is present. There is evidence that injury to the parent fiber may adversly affect the sprouting process.

It is the purpose of this paper to attempt to evaluate the effect of peripheral nerve crushing on the sprouting of afferent terminals into the spinal cord following cord hemisection. Such peripheral axon crushing can be considered to be both a source of injury and relative disuse to the parent nerve cell that produces the collateral sprouting.

The evidence for the above statements will be discussed in detail in the ensuing paragraphs, and a more detailed statement of the problem will follow the introductory review of pertinent literature.

INTRODUCTION

1- Characteristics of spasticity:

Magoun and Rhines(1) have defined spasticity as being a state of "increased resistance to manipulation, hyperactive deep reflexes, and clonus", with reflexes characterized by a "lowered threshold of the stretch reflex, and enlarged reflexogenic area, an augmented response, and a tendency to repetition, synchronization, and irradiation". They conclude that the basic feature of the spastic state is an exaggerated or hyperactive stretch reflex.

Spasticity as so defined is a special case of a more generalized phenomenon that occurs subsequent to injury or destruction of any part of the nervous system. This phenomenon is well described in Cannon's "Law of Denervation"; "When in a functional chain of neurones one of the elements is severed, the ensuing total or partial denervation of some of the subsequent elements in the chain causes a supersensitivity of all of the distal elements in the chain, including those not denervated, and effectors if present, to the excitatory or inhibitory action of chemical agents and nerve impulses. The super-sensitivity is greater for the links which immediately follow the cut neurones and decreases progressively for more distal elements"(24).

This hyperactivity or super-sensitivity of effector organs or neuronal pathways subsequent to nervous system lesions does not appear to always be the consequence of the same mechanism. It has variously been ascribed to "release" mechanisms(28)(1)(4)(6), "chemical sensitization" (15)(16), and to the "sprouting" of new pathways(17)(18). Each of these postulated mechanisms will be discussed in detail in following paragraphs.

2- Spasticity secondary to hemisection of the spinal cord:

Hemisection of the mammalian spinal cord is attended in the acute stage by an interval of ipsilateral areflexia or hyporeflexia aboral to the site of hemisection(19)(20)(21)(22). This interval of hyporeflexia, defined as "spinal shock" by Fulton in 1937,(22) has a duration of increasingly greater magnitude as one ascends the phylogenetic scale (7)(23). In addition to hyporeflexia the condition of spinal shock is associated with a concommitant interval of increased susceptibility to reflex inhibition(25). It has been well demonstrated that the changes occuring during spinal shock are to a major extent a consequence of the interruption of descending facilitatory influences that maintain the reflex arcs in a constant state of tonus(25)(26)(27). In particular interruption of the vestibulospinal tract seems to be of major importance in this respect although reticulospinal and pyramidal tracts probably play varing roles in the etiology of spinal shock depending on the phyologenetic station of the animal studied(22)(26)(27). Interestingly it has also been noted that there is a transient state of decreased excitability in spinal neurones following dorsal rhizotomy as measured by neuronal response to impulses in the corticospinal tracts(10).

Following the interval of spinal shock there is a gradual heightening of ipsilateral reflex activity over a period of two to four weeks. This is associated with clonus, and increased muscle tonus especially marked in extensor muscles(2)(7)(21)(23). The polysynaptic reflex threshold is markedly lowered on the hemisected side although the monosynaptic threshold is little altered(21).

Other functional changes resulting from cord hemisection include ipsilateral loss of voluntary movement, proprioception sense, and

spatial tactile discrimination. There is a thermal, pain, and touch defecit most prominent on the contralateral side. These changes are part of the Brown-Sequard syndrome(2)(5).

It is documented that the voluntary motor power loss secondary to cord hemisection is greatly diminished over a long period of time in primates, functional recovery being almost complete in some instances. It has been postulated that this is due to increased utilization of corticospinal fibers that cross below the hemisection site(20).

3- Theories of spasticity:

a) Unbalance between descending facilatory and inhibitory influences: "Release" and "Influx" theories.

Hughlings Jackson is accredited with being the first to clearly correlate certain kinds of spasticity with a "dissolution" of the influences of higher centers upon the neurones of the spinal cord. He noted that from both an evolutionary and functional standpoint the central nervous system can be fractionated into a hierarchy of levels, the lowest being the spinal cord, and the highest the associational areas of the cerebral cortex. He postulated that if the higher centers are separated from the lower the result is a consequence of two factors; first a release of the lower centers from the governing influences of the higher, and secondly a subsequent influx of facilitatory influences onto lower centers from unopposed intact higher centers(28). More recent work has supported Jackson's concept that certain types of spasticity are a consequence of the above postulated mechanism.

As noted above, Magoun and Rhines have postulated that the basic feature of the spastic state is an exaggerated or hyperactive stretch reflex

(1). The simple stretch reflex actually consists of a minimum of four reflexes: (a) facilitation from annulospiral endings upon ventral horn cells; (b) inhibition from Golgi tendon organs upon the alpha ventral horn cells; (c) inhibition from unknown endings upon the gamma ventral horn cells with consequent indirect removal of excitation upon the alpha motoneurones from annulospiral endings; and (d) myotube ending inhibition of extensors and facilitation of flexors(29).

It is established that the myotatic reflex in both its static and phasic components is controlled directly by the rate of firing of group Ia afferents from muscle spindle annulospiral receptor organs. These group Ia afferents end monosynaptically on alpha motoneurones confined almost entirely to innervation the strip of muscle containing the active annulospiral ending(30)(31). The rate of discharge in the annulospiral ending itself is linearly dependent upon the degree of passive muscle extension, or logrithmicaly related to the muscle load(32)(33). The frequency of the annulospiral ending discharge is also related to the activity of the gamma motor neurones which innervate the muscle spindle fibers(29)(32). The gamma motoneurones receive facilitatory and inhibitory influences from supraspinal sources, spinal cord proprioception arcs, and cutaneous afferents involved in the flexion and pinna reflexes(29)(32)(34)(35).

Thus although a hyperactive stretch reflex ultimately depends upon increased alpha motor neurone activity, the primary site of hyperreactivity does not necessarily have to be the alpha motoneurones themselves. For example a hyperactive gamma efferent system provides an equally favorable basis for exaggerated stretch reflexes. It is easily seen that an unbalance of descending influences could act at several

different spinal cord sites to produce exaggerated stretch reflexes. Indeed there is evidence to suggest that some types of spasticity are primarily the result of a hyperactive gamma system, while others are primarily a result of hyperactive alpha motoneurones independent of the gamma loop.

The former is exemplified by the Sherrington type of decerebrate animal characterized by extensor rigidity, hyperactive deep reflexes, and clonus which have been well correlated with appropriately increased gamma activity and which are abolished by dorsal root section, ie by interruption of the gamma loop(23)(29)(35). The latter is illustrated by the type of spasticity induced by the anemic decerebration of Pollack and Davis in which half of the cerebellum and part of the pons are destroyed(36)(37). In this preparation the spasticity is not abolished by dorsal root section, and the primary activity is directly in the alpha motor system, the gamma loop in this case behaves in a passive manner(29). The difference in the two types of spasticity seems to depend on the state of activity in the anterior cerebellum, since a gamma animal (Sherrington type of decerebration) can be converted to an alpha animal (Pollack Davis decerebration) by cooling or injuring this structure(29).

Several supraspinal structures have been associated with certain types of spasticity such as decerebrate rigidity that are secondary to an unbalance of supraspinal influences on the spinal cord. These will be discussed individually in succeeding paragraphs. In evaluating the relative balance of supraspinal influences on the spinal cord it is important to keep in mind that the role of any given supraspinal structure is a function of phylogenetic station, and that the higher the degree of encephalization

the more powerful are the influences of the higher centers relative to the lower(19).

The corticoreticulospinal inhibitory system:

Hines(39) in 1937, noted that extirpation of cortical area 4s resulted in symptoms of spasticity without voluntary motor paralysis. Activation of this strip of cortex produces inhibition of both resting tone, voluntary movement, and suppresses electrical activity of other parts of the cortex. Suppression of cortical electrical activity was found to be dependent upon a recurrent circuit between cortex and basal ganglia, but the latter structures were found to be unnecessary for inhibition of resting tone(40). The primary terminals of the fibers originating in this powerful cortical inhibitory region have been shown to terminate in the caudate nucleus and lower reticular formation(41).

The caudatoreticulospinal inhibitory system:

The projection from area 4s of the cerebral cortex has been shown to end in part in the striatum(40). In the intact brain and after degeneration of the internal capsule stimulation of the striatum relaxes or inhibits muscular contraction(42). Striatal ablation subsequent to cortical lesions causing spasticity greatly augments the intensity of the spasticity. It appears that the striatal inhibitory influences on spinal pathways is mediated through the reticulospinal system(14).

The cerebelloreticular inhibitory system:

Employing physiological stimuli to cutaneous, muscle and joint receptors it is possible to map an evoked response pattern in the anterior lobe and lobulus simplex of the cerebellum. This response is somatotopically the same for skin, joint, or muscle afferents. The tail is represented

in the lingula, the hindlimb in the centralis, the forelimb in the culmen, and the head and neck in the lobulus simplex(44)(45). Effector areas have the same somatotopic distribution as the receiving areas(46). Stimulation of medial cortical sites produces ipsilateral relaxation of extensor muscles in a decerebrate preparation, with concommitant contraction of flexors. Lateral cortical sites when stimulated cause ipsilateral increased extensor tonus and inhibition of flexors(47). It has been suggested that these cerebellar effector functions are mediated primarily through the gamma motoneurone system(48). Ablation of the anterior lobe of the cerebellum inactivates the supraspinal control of the gamma system and the site of supraspinal influence is shifted to the alpha system, suggesting that the cerebellum plays a very important role in organizing alpha-gamma linkage(29)(49). The inhibitory pathway from the cerebellum is relayed by way of the fastigial nucleus to the reticular formation(50).

The bulbar reticular inhibitory system:

A powerful, low threshold inhibitory mechanism has been demonstrated in the bulbar reticular formation by Magoun and Rhines(51). This mechanism is located in the ventromedial part of the bulbar reticular formation and extends forward to the trapezoid body. It appears that the cerebral and cerebellar inhibitory influences are mediated through the bulbar reticular formation, and thence to the spinal cord via the reticulospinal tracts(1)(14). Indeed the bulbar reticular formation per se seems incapable of intrinsic inhibitory activity(1). Activation of the inhibitory mechanisms acting through the bulbar reticular formation is accompanied by a corresponding decrease in gamma motor neurone discharge (29).

Vestibulospinal and reticulospinal facilitatory systems:

In the carnivore the extensor hypertonus of decerebrate rigidity can be abolished by destruction of Deiter's nucleus, or by interruption of the vestibulospinal pathway. This demonstrates the powerful facilitatory influence of the vestibulospinal pathways on the neurones of the spinal cord serving these muscles(26)(27)(52). As discussed in a previous section of this paper the vestibulospina system also appears to be strongly implicated in the etiology of spinal shock in the carnivore. The role of the vestibulospinal system in the primate is similar, but of a smaller magnitude than in the carnivore(1).

Magoun and Rhines have described a locus in the reticular formation outside of the reticular formation inhibitory site that when stimulated provides a facilitatory effect on pre-existing motor activity(53). This facilitatory brain stem system appears to take origin in the basal diencephalon where it receives contributions from the globus pallidus, midline, and other nuclei of the thalamus(1). It has also been demonstrated that stimulation of the facilitatory reticular formation site greatly augments the already hyperactive stretch reflexes present in a Sherrington type of decerebrate animal(54). It is documented that the facilitatory effects obtained by stimulating the reticular formation facilitatory site are exerted bilaterally, with crossing occuring both in the brain stem and in the reticulospinal pathway at the spinal cord level(55).

Thus it appears that the vestibulospinal and reticulospinal facilitatory pathways constitute two distinct systems contributing excitatory influences to the spinal cord "to some degree able to substitute one for the other". However, the vestibular pathway seems to

better maintain extensor tonus, while the reticulospinal pathway seems to contribute more to hyper-reflexia(1).

Certain types of spasticity are clearly related to an unbalance of the above noted supraspinal influences on the neurones of the spinal cord. Decerebrate rigidity induced by transection of the brain stem either through the caudal part of the diencephalon or at any mesencephalic level(7) can thus be explained by dissolution of the powerful cortico-reticulospinal and caudatospinal inhibitory systems while leaving the powerful vestibular and reticular facilitatory pathways intact(14). Spasticity of this type has two important distinguishing characteristics: (a) it has an abrupt onset(6)(23), and (b) it is abolished by restoring the relative balance of influences on the spinal cord neurones. For example decerebrate rigidity due to dissolution of the corticobulboreticular and caudatobulboreticular inhibitory systems while leaving the vestibulo-spinal facilitatory influences intact can be abolished by dissolution of the influences of the vestibulospinal system(27).

Spasticity such as that induced by transection or hemisection of the spinal cord in contrast to the above follows an interval of marked areflexia and flaccidity as discussed earlier in this paper. Furthermore, spasticity due to hemi or transection of the cord cannot be abolished by restoring the relative balance of descending influences. For example, if cord hemisection is followed by complete cord transection after an interval sufficient to induce spasticity on the hemisected side, the spasticity remains on the previously hemisected side. The previously normal side now falls into a state of spinal shock that is gradually replaced by a state of spasticity(22). These considerations make it necessary to invoke other theories to account for the spasticity due to cord hemisection.

b) Chemical sensitization by denervation:

In 1880, Claude Bernard expressed the opinion that, "The excitability of all tissues seems to augment when they are separated from the nervous influence which dominates them"(59). The first phenomenon recognized in connection with chemical sensitization following denervation were the so called "paradoxical pupillary dilatation", and the "Phillipeaux Vulpian" phenomenon.

The "paradoxical pupillary dilatation" phenomenon was first described by Budge in 1855(56). He noted that if in a rabbit the preganglionic sympathetic fibers to one iris and the postganglionic fibers to the other iris were interrupted at the same operation, the pupil was more dilated on the side deprived of the postganglionic pathways. Other investigators reported that following preganglionic denervation, asphyxia, ether anesthesia, or indeed any cause of excitement or distress caused more marked pupillary dilatation on the denervated side. This of course represented a paradox in terms of what was expected following severence of the nerve supply known to be responsible for pupillary dilatation. Early theories of explanation considered the paradox to be the consequence of weakened sphincter muscles rather than an exaggerated contraction of radial fibers. This idea was disproved by showing that eserine which causes a contraction of the sphincter muscles of the iris caused a more profound contraction on the sympathetically denervated side(57). The riddle was solved when Metzer and Auer(58) demonstrated that subcutaneous injections of adrenalin elicited the paradoxical response. Furthermore, if an adrenalectomy was performed prior to the test the paradoxical response would not be obtained. Thus the paradoxical pupillary response was given a reasonable explanation as being due to an increased secretion of adrenalin resulting

from emotional excitement, acting with special effectiveness on the denervated retractor muscle of the iris(15).

The Phillipeaux-Vulpian phenomenon which consists of an anomalous response of denervated striated muscle to stimulation of nerves distributed to blood vessels was similiarly explained on the basis of a supersensitivity of the denervated muscle to the minute amounts of AcCh liberated at the terminals of the blood vessels(15).

Many other examples of effector organ sensitization following denervation have been described. The blood vessels of the rabbit ear are supersensitive to adrenalin following denervation, as are the pilomotor muscles of the head and neck. The uterus and intestine have an increased susceptibility to the inhibitory or relaxing effects of adrenalin following denervation. The bronchioles show far greater relaxation when subjected to a challenging dose of adrenalin following chronic sympathetic denervation(16).

The early work on chemical sensitization of neurons was carried out on the sympathetic nervous system. Elliot in 1907, called attention to the resemblance between the phenomena at the junction between motor nerves and skeletal muscle, and at the junction between preganglionic fibers and nerve cells in sympathetic ganglia. In both places nicotine is stimulatory and in large doses is paralytic in action. In both places curare can block the action or passage of nerve impulses. When chemical mediation of nerve impulses was demonstrated another resemblance was disclosed, for acetylcholine appears to be the mediator at the two junctions(59).

Cannon and Rosenblueth in 1936, undertook to study the sensitization of a sympathetic ganglion by preganglionic denervation. The nicitating

membrane of a cat was employed as an indicator. The preganglionic fibers in the cervical sympathetic supply were aseptically severed on the right side and at least a week was allowed for degeneration. The preganglionic fibers on the left side were freshly cut at the time of the terminal experiment and both nicitating membranes were arranged for recording. The adrenal glands were removed to prevent possible contractile effects of adrenalin on the nicitating membrane. It was found that the chronically denervated side was markedly more sensitive to AcCh. This must represent sensitization of the ganglion itself since the nicitating membrane is not effected by AcCh directly either in the normal or chronically denervated state(13).

The sensitizing of ganglion cells by denervation naturally suggested that the nerve cells of the brain and spinal cord might also be chemically sensitized if denervated, that is deprived wholly or in part of the nervous connections from which they routinely receive impulses. Cannon and Haimovici investigated this point employing spinal cord hemisected cats. The response of the quadriceps muscles to intraortic injections of AcCh was measured. The aorta was occluded at a point just above the common iliacs to prevent direct effects of the drug on the muscle. If at least two days was allowed after the hemisection operation it was found that the quadriceps contraction was far greater on the hemisected side(60).

That lesions of the brain are also followed after an appropriate interval by chemical sensitization of the resultant partially denervated neurones also has been documented(16)(61)(62).

It has been found that the chemical sensitivity of denervated neurones is not restricted to AcCh and related compounds, but indeed a great variety of analeptics and also depressants have a greatly enhanced

effect on "denervated" neurones(16).

Partially denervated neurones also have a supersensitivity to impulses reaching them from remaining presynaptic terminals. Sympathetic ganglion cells are sensitized to incoming impulses when partially denervated(12). Partial denervation of spinal neurones consequent to intradural section of dorsal roots renders these neurones more responsive than normal to the excitatory action of nerve impulses reaching them from higer centers. A deafferented limb of a cat exhibits surprising hyperactivity when vestibular discharges are initiated by appropriate movement of the animal with respect to its surroundings(63).

In spinal cord hemisected animals the extensor and flexor myotatic reflexes are greatly increased on the ipsilateral side. Also the inhibitory effects of ipsilateral afferent nerve volleys on knee jerks was frequently either greatly prolonged or greatly shortened in duration of effect(16).

Cats subjected to extensive unilateral dorsal root section have an increased reactivity to descending impulses on the ipsilateral side after an interval of about five days of hyporeactivity(10).

Many theories have been suggested to account for chemical sensitization secondary to denervation. Relative "disuse" per se of the denervated structures has been largely discounted because of multiple examples of denervated structures that become sensitized despite a high rate of continued activity(15). Early theories postulating increased storage of the transmitter substance within the peripheral cell have been discounted by the finding that the mediators are produced in presynaptic elements rather than in the postsynaptic cell(64).

It has been suggested that supersensitivity might be due to a

decreased rate of destruction of the stimulating substance. It has indeed been found that the concentration of acetylcholinesterase is decreased in denervated structures. This is true for both peripheral effector organs and central nervous system tissue(65)(66)(67).

However, even if it be firmly established that there is a retardation of transmitter substance destruction in denervated structures this cannot account for hypersensitivity to chemical agents other than transmitter substances(16).

It has been postulated that supersensitivity is a consequence of altered membrane permeability. Sensitized muscle has an increased amount of extracellular fat and calcium and a decreased concentration of intracellular glycogen and creatinine(68). The potassium content of sensitized muscle seems to be normal although denervated muscle is more permeable to that ion(69)(70)(71).

A study of electrical potentials in muscle led Li(72) to the conclusion that spontaneous fibrillation potentials do not originate in specific focal areas in muscle fibers, but are due to differences in membrane properties of these fibers which are associated with changes in metabolic activity. Electrical changes included a decreased resting membrane potential and a tendency toward oscillation of the potential. Hand in hand with these changes in membrane electrical activity there is an increased hexokinase and decreased cytochromeoxidase concentration in denervated muscle(73)(74).

Clearly both "release" and "chemical sensitization" phenomena can lead to a hyperactivity or hyper-responsiveness of neuronal pathways, and thus explain the hyperactive stretch reflex that Magoun and Rhines define as "the basic feature of the spastic state". However, that

"release" phenomena per se cannot account for certain types of spasticity such as that induced by transection or hemisection of the cord has already been discussed. "Chemical sensitization" also falls short of being a completely satisfactory explanation for certain types of spasticity. It is documented that partially or totally denervated neurones are reinnervated either by regeneration of the sectioned axons or by sprouting of collaterals from adjacent intact axons. In these cases as the reinnervation process progresses, the chemical sensitization declines and may finally disappear(17)(18). However, a state of hyper-responsiveness still exists in the now reinnervated neuron(17). The concept of collateral sprouting will be discussed in the succeeding paragraphs.

c) Collateral sprouting following partial denervation:

Probably the first documented description of the formation of new processes or collaterals by intact axons lying adjacent to degenerating axons following injury to the central nervous system was provided by Ramon Cajal(76). Subsequently collateral sprouting by intact fibers following partial denervation has been described in both peripheral and central nervous system structures.

Wedell, Gutmann, and Guttmann have described experiments in which they sectioned the sural nerve of the rabbit. Within 24 hours they could detect a decrease in the resultant area of sensory deficit as measured clinically. Histological demonstration of sprouting of collaterals by intact adjacent axons into the denervated zone is possible one week following the nerve section(77).

Collateral sprouting of intact axons following partial denervation of muscle is well documented. Two weeks following denervation sprouts from adjacent intact axons may be demonstrated histologically. The

sprouts arise within the intramuscular portions of the intact nerve fibers and travel preferentially, down degenerating nerve sheath pathways(78). Sprouting may occur either at nodes of Ranvier, or at internodal sites(78) and are proportional in number to the number of end plates vacated by the degenerating fibers(79). The sprouts establishing end plate connections become functional(84). Those failing to do so appear to regress(79).

The stimulus to sprout seems to be provided by a humoral agent(80)(81)(82)(83). More specifically there is evidence that the substance that stimulates axon collateral sprouting is a moderately unsaturated fatty acid possibly related to the 18 carbon cis-vaccenic acid. It is postulated that this substance is released by degenerating myelin and/or Schwann cell activity and diffuses to adjacent intact axons, disrupting the axolemma and bringing about the first stage of sprouting(79)(81).

A marked increase in the number of sprouted collaterals from intact fibers in paretic rat muscle is noted following electrical stimulation of the lumbar cord or lumbar plexus for an appropriate interval. This is thought to be the result of an enhanced synthesis and increased peripheral flow of axoplasm(75)(83). This is supported by finding of accelerated peripheral displacement of P32 in frog nerve fibers following electrical stimulation(85), and by evidence that cytoplasmic antimitotic drugs retard sprouting whereas nuclear antimitotic agents do not(83).

It has also been noted that Pyronin, a dye related to Methylene Blue, stimulates sprouting of collaterals. Methylene blue and other closely related molecules do not seem to possess this property. The mechanism of action of Pyronin remains to be elucidated(83).

It seems to be established that regenerating or sprouting nerve fibers are not confined to reinnervating muscles of their original motor unit or functional synergists thereof. Crossed nerve experiments indicate that a given motor nerve will reinnervate a muscle that previously was antagonistic in function to the muscle originally served by that motor nerve(86)(87)(88). However, sensory nerves if implanted into a denervated muscle will not establish functional end plate connections although they show some inclination to sprout(84).

The occurance and function of collateral sprouting in the sympathetic nervous system was described in a paper of the same title by Murray and Thompson in 1957(17). Employing adult cats they unilaterally severed the T1, T2, and T3 gray and white rami communicantes. This resulted in the degeneration of 90% of the fibers in the cervical sympathetic trunk at its point of entrance into the superior cervical ganglion. The three rami severed supply most of the fibers innervating the nicitating membrane, and all fibers innervating the radial muscles of the iris in the normal cat. By stimulating the cervical sympathetic trunk just caudal to its entrance into the superior cervical sympathetic ganglion they could observe and compare the resulting responses of the nicitating membrane and pupillary dilator muscles on the partially denervated and control sides of the animal. They also studied the resultant chemical sensitization by injecting acetylcholine and other drugs into the carotid arteries.

Four days following the denervation procedure they found that there was a prominent chemical sensitization of the partially denervated structures. The response of the nicitating membrane to cervical sympathetic trunk stimulation however remained much less on the partially

denervated side than on the control side. There was no evidence of pupillary dilatation on the partially denervated side.

At eighteen days there was an even more pronounced chemical sensitization of the partially denervated structures. However, stimulation of the superior cervical sympathetic trunk now provided a much greater response of the nictitating membrane on the partially denervated side than on the control side. Mild pupillary dilatation was now noted on the partially denervated side with stimulation of the sympathetic trunk.

By four weeks the chemical sensitization had virtually disappeared. However, the response of the nictitating membrane to stimulation of the superior cervical sympathetic trunk was still much greater on the partially denervated side. In addition the pupil on the partially denervated side now responded with a nearly normal dilatation following stimulation of the sympathetic trunk on that side.

Histological demonstrations revealed the onset of collateral sprouting from the remaining 10% of the fibers within the sympathetic trunk within five days following the denervation procedure. Sprouting also occured from the intact terminal within the superior cervical sympathetic ganglion. By the fourth week many of these sprouted terminals had established normal ending on ganglion cells, and by the eighth week maturation was complete. It was demonstrated that the return of function was definitely not due to regeneration of the previously sectioned fibers.

In spite of the reinnervation of the cells in the ganglion, perfusion studies revealed that total AcCh release following stimulation of the superior cervical sympathetic trunk only achieved 60% of the control

side value. Hexamethonium was correspondingly more efficient in blocking the partially denervated ganglion. The authors postulate that this represents a limited capacity for the parent nerve cell to provide AcCh for terminals. The amount of AcCh liberated was the same at 21 and 100 days following the denervation procedure.

Guth and Bernstein have recently provided some evidence that there may be selectivity in the re-establishment of synapses in the superior cervical sympathetic ganglion following partial denervation(89). This study was as follows: The T1 rami provide pupillary dilatation fibers whereas the T4 rami normally do not. If T1 is crushed T4 stimulation does provide pupillary dilatation 30 days later. As discussed in the preceding paragraphs this interval is sufficient to abolish chemical sensitization. Six months later T1 again causes pupillary dilatation, apparently having regenerated, but T4 now does not. These authors conclude that T4 sprouts and fills in the vacancies on the ganglion cells vacated by the degenerating T1 fibers. However, T1 has preference and when it regenerates it replaces the sprouted T4 fibers which apparently regress.

Within the central nervous system intraspinal sprouting of dorsal root axons has been well demonstrated by Liu and Chambers(90). These authors employed two major series of cats. One series had extensive unilateral dorsal root section proximal to the tract. At the time of terminal experiment an intact dorsal root was sectioned on both the experimental and control sides, and 4-5 days later its degeneration pattern was determined histologically employing modified Nauta stains specific for degenerating axons. The terminal experiment followed the initial operation by about 260 days, an interval necessary for complete

disappearance of degeneration debris from the initial operation.

It was found that the intact dorsal root on the experimental side had sprouted profusely. The sprouting elicited by the extensive adjacent dorsal root section was most consistent in the dorsal horn and around Clarke nucleus. Occasionally significant sprouting was also demonstrated in the ventral horn and around the intermediate nucleus of Cajal. Sprouting elicited by intracentral pathway section was greatest when the bulbar pyramid was sectioned as opposed to sigmoid gyrectomy, and sprouting was the greatest on the opposite side in the low cervical and upper thoracic segments.

To establish whether or not unilateral denervation of the spinal cord stimulates only the axons on that side to sprout, or involves axons of the opposite side as well, the following experiments were performed. Extensive unilateral dorsal root section was followed in 277 days by section of a single contralateral dorsal root. The axonal debris was limited to the side of the cord on which the single dorsal root was severed. Thus sprouting across the midline apparently did not occur.

The possibility that contralateral sprouting had occured but had not crossed the midline was considered and rejected since in cats that had but a single dorsal root sectioned the resulting axonal debris was almost identical in distribution and quantity with that observed on the nondenervated side of the experimental animal. Edds also has described the failure of sprouting across the midline following unilateral denervation of the diaghram(79). It is postulated by Liu and Chambers that the failure of sprouting across the midline is probably not due to a physical barrier but rather may represent some specificity of terminal fields or of degeneration products. The quantity of sprouting

seems to be proportional to the amount of denervation. This conduns well with previously described findings of studies of sprouting in peripheral muscle nerves.

Liu and Scott(91) in studying regeneration in the dorsal spinal cerebellar tracts in the cat have reported that in animals with unilateral isolated lesions of the dorsal spinal cerebellar tracts regeneration occured only to a minor extent. However there were occasional instances in which conducted potentials were found in the dorsal spinocerebellar tract above the lesion on the operated side in response to stimulation of the contralateral dorsal roots or dorsal spinocerebellar below the lesion. This was in contrast to normal cats and the authors postulate the possible sprouting of a new conductile pathway between the right and left dorsal spinocerebellar tracts above the level of the lesion.

In 1958 Austin, McCouch and Liu and Liu(13) reported evidence that the presynaptic component of the intermediary cord potential previously shown to be due to afferent terminals(92) is increased on the ipsilateral side of hemisected cats and monkeys. Lesions confined to the dorsal columns also induced sprouting of afferent terminals but these failed to establish terminal connections and apparently regressed. The increased afferent terminal potential was correlated with histological studies demonstrating sprouting of the terminals.

It was noted in as early as 1828 that nerve crossing experiments in which peripheral nerves to antagonistic muscles were cross united (Flourens-1828, Bawa-1885, Spitzy-1905) resulted in functional reorganization on a central nervous system level so that nearly normal coordinated function often returned with the passage of time(38). In Cichlid fishes for example crossing of the nerve to the jaw depressor is followed after an interval of time with the return of normal function

well coordinated with the opposite side(93).

Recent evidence by Eccles et al(86)(94)(95) has revealed that sprouting of fibers surviving retrograde chromatolytic death onto appropriate motoneurones may account for some of this apparent central nervous system reorganization. They found that after crossed nerve experiments, for example after crossing the nerve to the peroneus muscles with the nerve to the medial gastrocnemius, there was a marked increase in monosynaptic EPSP activity recorded in peroneus motoneurones when stimulating the nerve to the medial gastrocnemius that now innervated peroneus muscles. Interestingly the converse was not true. That this apparent shift in terminal afferents from medial "gastroc "to peroneus motoneurones was not the result of random sprouting was established by a study in which the "gastroc "and peroneus nerves were sectioned and self reunited. In this case there was no increase of monosynaptic EPSP activity recorded in peroneus motoneurones when the nerve to the medial "gastroc " was stimulated. The authors conclude that there is a specificity in determining terminal connections, in a given group Ia afferent fiber can only make functional connections with motoneurones innervating the same muscle that the Ia fiber arises from. Furthermore there was evidence that the preoperative connections with their motoneurones now tended to regress. As described above other authors have also presented evidence that sprouted terminals have a certain specificity in selection neurones with which to establish synaptic connections(79)(89)(90).

Purpura and Housepian have recently reported findings that if immature neocortex is isolated subpially there is a conversion of type one pyramidal neurones into type two aroiform elements with multiple

axon collaterals. This event coupled with the normal postnatal maturation of apical and basilar dendritic systems results in a marked increase in synaptic activity initiated by weak surface stimulation. The analyses of surface and intrafocal potentials and the effects of topically applied aminoacids permit distinctions between spreading bursts and local repetitive paroxysmal discharges. Reorganization of intracortical synaptic pathways rather than denervation sensitization is inferred to be the major factor responsible for the development of hyperexcitability in isolated immature cortex(97).

CORD DORSUM POTENTIAL

Gotch and Horsley in 1891 were the first to report the presence of electrical potential alterations in the spinal cord during activity, employing as a measuring device a string galvanometer. However it remained for Gasser and Graham(98) and Hughes and Gasser(99) in 1933 to undertake the task of assigning form and significance to the potential changes recorded from the cord dorsum following stimulation of dorsal roots. Gasser and Graham described an initial triphasic spike of duration 0.5 Msec followed by a more prolonged negative potential with crest 2.2 Msec following the onset of the initial triphasic spike, and lasting about 10.2 Msec. Following this prolonged negativity these authors described a positive slow wave of lesser amplitude that crested about 20 Msec following the onset of the triphasic spike and lasted from 80 to 100 Msec. They found that the initial triphasic spike was resistant to asphyxia and repetitive stimulation and showed no signs of convergance when adjacent roots were stimulated. They calculated the triphasic spike to be temporaly compatible with an origin in fibers of conduction velocity of about 30 M per second. In light of these findings the authors concluded that the spike had its origin in the intramedullary course of the dorsal root fibers and termed this component of the cord dorsum potential the intramedullary spike, hereafter referred to as the IMS in this paper.

In contrast to the IMS the authors found that the slow negative component (termed the N wave and hereafter referred to as such) was very sensitive to as hyxia, was sensitive to repetitive stimulation, and showed signs of convergance when adjacent roots were stimulated. Since Sherrington had already long since attributed the phenomenon of convergance to a

"sign of post synaptic activity"(100), it was concluded that the negative wave had its origin in post synaptic structures, probably interneurones since antidromic stimulation of ventral roots did not produce an N wave as recorded from the cord dorsum.

The positive wave (termed the P wave) which terminated the cord dorsum potential was found to behave similiarly to the N wave and was also attributed to post synaptic structures by these authors.

Hughes and Gasser in 1934(99) confirmed the above findings and contributed the following additional information. The IMS has a longitudinal distribution along the cord dorsum of about 1.5 cm caudal to the point of entry of the stimulated root and many centimeters cephalad to the point of entry of the stimulated root if the recording electrode is placed over the dorsal columns. The IMS has its maximal amplitude directly over the point of entry of the stimulated root. The N wave actually has a bactrian form with a second peak occuring on the declining slope of the initial N deflection. The longitudinal distribution of the first of these components of the N deflection was measured to be 1.5 cm cephalad and caudal to the point of entry of the stimulated root. The authors termed the first of the negative peaks of the N wave N1 and the second N2. They will hereafter be referred to as such in this paper.

Hughes and Gasser in 1934(101) studied the response of the spinal cord to two afferent volleys, and by measurements of the N wave determined that the period of absolute refractoriness of interneurones is 10 Msec, with a period of relative refractoriness of about 2000 Msec. This correlated well with measurements of inhibiton of the flexor reflex and these authors concluded that inhibition of the flexor reflex is simply due to

block of interneurones.

The cord potential was studied during spinal shock in the cat and monkey by Stewart, Hughes and McCouch in a series of three papers in 1940(103)(104)(105). They found that the N wave threshold was increased in these animals following acute transection of the spinal cord. This elevation of threshold was found to last about one hour in the cat and about six hours in the monkey. The ventral root reflex remained depressed for a longer period of time thus suggesting that motoneurones per se are effected by spinal shock. The authors also found that in chronicaly cord hemisected animals the ipsilateral cord potential had a greater longitudinal distribution than the control side following acute mid-thoracic transection of the cord.

The N1 component of intermediary cord dorsum potential was further divided into two separate components by Austin and McCouch in 1955(92). These authors described a small independent wave on the ascending limb of the N1 potential that displayed resistance to asphyxia and repetitive stimulation and did not show signs of convergence with stimulation of adjacent roots. This suggested a presynaptic origin for this early N1 component in contrast to the later component of the N1 potential. Studies of electrical fields within and around the cord further indicated that this presynaptic component of the N1 potential had its origin in afferent terminals of dorsal root fibers. This early presynaptic component of the N1 potential was termed the N1a deflection, and the remainder of N1 was termed the N1b deflection. The N1a component was later shown by the same authors to be increased following chronic hemisection of the cord in cats and monkeys, a finding that correlated with histological demonstration of sprouting of the afferent dorsal root fibers following chronic hemisection(18).

Lindblom and Ottosson in 1956 studied the bulbar influences on spinal cord dorsum potentials and ventral root reflexes by stimulation of the bulbar reticular formation simultaneous with elicitation of cord potentials and ventral root reflexes in nembutalized cats. These authors found that stimulation of the retculospinal centers depressed the N1b component of the cord potential leaving the IMS and N1a components unaltered(106). An earlier paper by these authors had demonstrated that the N1b component is elicited by stimulation of low threshold cutaneous fibers and is enhanced by section of the spinal cord. When N2 was present however section of the cord abolished the N1b deflection suggesting that a suprasegmental facilitatory pathway also existed that functioned only when the inhibitory pathways from the suprasegmental levels were not active. Hemisection of the spinal cord affected both ipsi and contralateral N1b potentials suggesting a segmental crossing of the descending pathways influencing N1b(107). The authors found that the ventral root reflex could be inhibited with or without alteration of the N1b component, and furthermore that the ventral root reflex could be facilitated with concurrent depression of N1b. They concluded that the reticulospinal pathway provides inhibition at the segmental sensory relay of impulses to suprasegmental levels of impulses from low threshold skin afferents.

The anatomical site of origin of the N1b deflection was demonstrated by Lindblom and Ottosson in 1953(108). They found that lesions in the spinal cords of cats (lesions produced with osmic acid) altered the N1b potential only if the lesions were in the apical portion of the dorsal gray. Lesions in the dorsal lateral columns, intermediate gray and ventral horn did not alter the N1b potentials. Lesions in the basal portion of the dorsal gray affected the N1b deflection to a small extent if N1b was measured at its maximum crest value.

Bernhard in a paper describing the spinal cord dorsum potentials in relation to peripheral source of stimulation(109) found that the Nlb deflection had origin from stimulation of low threshold cutaneous fibers. He found that the Nlb deflection had maximum amplitude with only 70% maximum stimulus to the afferent fibers, a fact that suggests a more powerful articulation of the afferent fibers with the Nlb interneurones than have the afferent fibers controling the monosynaptic ventral root reflex. They also found that Nlb was very resistant to narcotics relative to the ventral root reflex, and that the ventral root reflex could be enhanced by an antidromic volley in the dorsal columns at a time when the Nlb deflection was blocked by the antidromic volley. Intraspinal recordings during these experiments indicated that Nlb was maximal at a point 1-2 mm below the cord surface in the dorsal gray. The Nla component was described following stimulation of low threshold muscle afferents. Stimulation of high threshold muscle afferents produced a late negativity (of latency compatible with the N2 deflection). Separate stimulation of cutaneous, high threshold muscle afferents, and low threshold muscle afferents were all followed by positive waves of different characteristics suggesting a complex origin of the so called P wave of the cord dorsum potential.

DORSAL ROOT REFLEX

The occurance of centrifugal potential changes in the dorsal root following a centripetal volley was first described by Gotch and Horsley in 1891(119). Since this observation was in opposition to the law of Bell and Magendie the finding was viewed with suspicion and largely disregarded until it was redemonstrated by Matthews in 1934(120). Toennies in 1938(119)(121) described some of the characteristics of this dorsal root reflex (hereafter abbreviated DRR) noting that it had an onset latency of about 4.0 Msec and duration of about 20-30 Msec, and displayed characteristics of summation, facilitation, and inhibition. A contralateral component was also described by Toennies in this paper.

Frank and Fuortes in 1955(122) demonstrated that a muscle afferent volley depresses the size of the monosynaptic EPSP produced by a volley in different afferent fibers, with no evidence of IPSP activity. This depression coincided well with the previously observed temporal characteristics of the DRR, ie it had its onset at about 5 Msec and slowly decayed. Total duration of the effect was sometimes as long as 200 Msec. Eccles et al (123)(124) extended Frank and Fuortes observations and noted that group Ia and Ib muscle afferents are potent sources of the DRR, especialy in knee and ankle flexor muscles, but that muscle afferent groups II and III are not sources of the DRR. Eccles postulates in the latter paper that the EPSP depression occuring without evidence of IPSP activity is the result of terminals ending on the presynaptic afferent terminals which a)depress the incoming action potential and thus reduce the amount of liberated transmitter substance and b) generate centrifugal impulses over the afferent fibers thus accounting for the DRR.

RETROGRADE CHROMATOLYSIS

Retrograde chromatolytic changes in ventral horn nerve cell bodies following section of their axons was first described by Nissl in 1892(110). Although there is disagreement as to the extent of the chromatolytic process in dorsal root ganglion cells following section of their axons peripheral to the cell body, there is no question that it does occure to some extent (111)(116).

The process of retrograde chromatolysis involves dissolution of the "Nissl" bodies normaly dispersed throughout the cytoplasm of the neuron soma(111)(113). It has been shown that the Nissl bodies are composed of folds of convoluted membranes (endoplasmic reticulum)(114) associated with clusters of granules now known to be RNA particles(110). The Nissl bodies bear a striking resemblance to structures in the cytoplasm of gland cells that are intimately concerned with synthesis(114).

The phenomenon of chromatolysis does not seem to follow an all or none basis. Following axon section by a period of days or weeks examination of a group of cells such as in a dorsal root ganglion or hypoglossal nucleus there is a spectrum of changes ranging from mild dissolution of the Nissl substance to more marked dissolution associated with perinuclear clumping of remaining Nissl bodies. More severe chromatolysis is associated with almost total dissolution of the Nissl substance and other evidence of disturbed cellular function, ie marked eccentricity of the nucleus (110)(111)(112).

The chromatolytic cycle has been studied in the hypoglossal nucleus of the rabbit with respect to RNA concentration, cell volume, cell mass by means of X-ray microradiograph at varying time intervals(112). In this study the regeneration period was divided into three intervals.

The first interval which lasts for about two days is called the latent period. During this period there is degeneration of several mm of the central part of the fiber and production of transient protoplasmic strands that later regress. During this period the cell volume remains constant, both the amount and concentration of RNA is constant, however the dry cell substance decreases by 40%.

The second interval is called the outgrowth period and lasts from day three to about day twelve. During this time the axons sprout and eventualy join the motor end plate. From the third to the sixth day the cell volume increases by 260%. The RNA fails to increase at this papid pace and the RNA concentration decreases by 60% during this period. The total cell protein during this period is somewhat increased.

The third interval is termed the maturation period and lasts from day 13 to about day 90. This is characterized by a prominent increase in axon diameter. The cell volume remains maximal until about day 40. Also at day 40 the RNA per cell is more than twice the normal value. However the RNA concentration remains somewhat low. Other proteins and lipids are also maximal at 40 days.

From this study it was calculated that during the total regeneration cycle the cell replaced 50 times the original cell quantities of organic cell material. The RNA per cell does not increase until the maturation period, but the protein per cell starts to increase with the outgrowth period; the authors postulate on this basis that chromatolysis represents the activation of existing RNA polymers.

Eccles et al(117) contend that careful extradural section of dorsal roots peripheral to the ganglion causes a minimum of injury explainable on the basis of damage done directly to the ganglion at the time of surgery. They state however that there is a 10% reduction

in fiber size in the dorsal root proximal to the ganglion. These authors conclude that extraganlionic section of dorsal roots is a valid way to study the effects of disuse on afferent pathways. This study does not state whether or not electrophysiological changes were noted in the presynaptic components of the reflex pathways studied and cites only changes recorded from the post synaptic elements, ie the ventral root reflex.

Hare et al (111) in contrast to the above, described abundant examples of chromatolysis in the dorsal root ganglion of the cat following section of dorsal root fibers distal to the ganglion. The percentage of cells showing evidence of chromatolytic change was not stated.

A study by Eccles et al(118) on chromatolyzed ventral horn cells following section of ventral roots from 14-44 days has demonstrated that ventral horn cells in a state of chromatolysis show no change in resting potential, spike potential, or after potentials. EPSPs recorded from these cells however exhibited a significant reduction in size and rise time. The EPSPs also demonstrate abnormal superimposed "hump like depolarisations" which are abolished by hyperpolarization. The authors attribute this to abnormally excitable patches of membrane on the soma and dendrites.

STATEMENT OF PROBLEM

In the foregoing paragraphs a definition of spasticity has been discussed, and the state of unilateral spasticity induced in cats by ipsilateral spinal cord hemisection described. The three theories of spasticity have been discussed in order of their chronological origin, and their relative merits described with reference to spasticity secondary to spinal cord hemisection. It has been pointed out that a simple unbalance of descending influences does not by itself adequately account for certain characteristics of spasticity subsequent to spinal cord hemisection. In particular it does not afford an explanation for the chronological order of events following spinal cord hemisection; that of profound ipsilateral spinal shock that is gradualy replaced by a state of ipsilateral spasticity. This is in contrast to spasticity induced by decerebration procedures which is of immediate onset and is apparently well accounted for by a simple unbalance of descending influences.

The second theory of spasticity proposed, that of chemical sensitization following partial denervation of either effector organs or neurones has been discussed. It has been pointed out that this phenomenon provides a better explanation for the chronological chain of events following spinal cord hemisection. However studies have been cited that indicate that in certain instances both with respect to peripheral effector organs and central nervous system neurones the sprouting of adjacent intact axons reinnervate the partialy denervated structures. When this occures the chemical sensitization concommitantly dissapears. In particular with reference to the spinal cord sprouting of adjacent dorsal root afferents has been described following spinal cord hemisection. This is associated

with an increase in that component of the cord dorsum potential that has been related to dorsal root afferent terminals. At present there is no direct evidence that the sprouted terminals establish functional connections.

As described in earlier paragraphs of this paper several studies have demonstrated that following partial denervation of peripheral structures the sprouting of adjacent intact axons can be influenced by several procedures. Electrical stimulation of the intact fibers increases the amount of sprouting; this is thought to be due to an enhanced synthesis and increased peripheral flow of axoplasm. This hypothesis is supported by findings of accelerated peripheral displacement of P32 in frog nerve fibers following electrical stimulation, and by evidence that cytoplasmic antimitotic drugs retard sprouting.

It is reasonable to postulate that a similiar disturbance in the cytoplasm of dorsal root axons might also alter their capacity to sprout into the spinal cord following partial denervation of spinal neurones by spinal cord hemisection. As will be discussed in succeeding paragraphs crushing the dorsal root fibers at a point peripheral to the dorsal root ganglion institutes both changes in the metabolic functions concerned with cytoplasm production, and in the relative state of "use" of the fibers. If this alters the sprouting process of the dorsal root fibers this fact should be reflected in the cord dorsum potentials related to dorsal root afferent terminals. Further if sprouting of dorsal root afferent terminals plays a role in the spasticity subsequent to spinal cord hemisection there should be a change in the clinical pattern of spasticity.

The hypothesis of this paper may then be stated as follows: peripheral crushing of dorsal root axons simultaneous with spinal cord hemisection will decrease the capacity of the dorsal root fibers to sprout onto the spinal neurones partialy denervated by the hemisection procedure.

This will result in a decreased presynaptic terminal potential of the cord dorsum potential relative to the increase previously described subsequent to cord hemisection. Further, if the sprouted afferent terminals are directly related to the spasticity induced by spinal cord hemisection the pattern of spasticity should be altered or abated if the N1a potential is depressed by the nerve crush procedure.

METHODS AND MATERIALS

The experimental animals were divided into two major groups; 1) an acute control group subjected only to the terminal experiment as described below, and 2) chronic animals divided into six subgroups as determined by various chronic experimental procedures as described below, and the interval of time elapsing between the experimental lesion and the terminal experiment.

Hemisected animals

As discussed in the introduction to this paper a state of relatively unilateral spasticity can be induced in the lumbosacral cord of the cat by chronic mid-thoracic hemisection of the spinal cord. The mid-thoracic hemisections were performed as follows. The mid thoracic vertebral column was exposed through a two centimeter dorsal midline incision performed under ether anesthesia. The cord and meninges were exposed between vertebral bodies with rongeurs. One limb of a sharp pair of jewelers forceps was inserted through the dura and cord in the midline until the underlying bone was probed. The other limb was directed ventrally extradurally just lateral to the cord and coverings. The cord interposed between the two limbs of the forceps was then pinched firmly for about fifteen seconds. Repair of the tissues overlying the cord was effected with 2-0 gut for deep tissues and 2-0 silk for cutaneous tissues. Following the terminal experiment histological studies were made of the hemisected area to determine the accuracy of the lesion. In this group ten animals were employed of 30 days chronicity (interval between experimental lesion and terminal experiment) and ten animals of 90 days chronicity.

Denervated animals

As discussed in the introduction to this paper, denervation as effected by crushing a peripheral nerve gives rise to two types of changes in neuronal pathways proximal to the lesion. In the afferent fibers there is a resultant interval of disuse of the proximal neuronal pathways involved. In the neurones involved there is a process of retrograde degeneration apparently brought about by the fiber injury and by the process of fiber regeneration.

In this group of animals unilateral denervation of a hind limb was accomplished by crushing the sciatic, obturator and femoral nerves. The sciatic nerver was exposed and crushed at its exit through the greater sciatic notch, the obturator nerve at its exit through the obturator foramen so as to include both anterior and posterior divisions, and the femoral nerve at its exit into the femoral canal. Crushing was performed with a hemostat, the resultant lesion being about 2-3 mm in width. The adequacy of the lesion could be determined visualy at the time of the procedure. In addition clinical examinations were employed to check the completeness of nerve interruption (see below). In this group there were ten animals each of 30 and 90 days chronicity.

Hemisected and denervated animals

Hemisection and ipsilateral denervation procedures were carried out concomitantly as described above. In this group there were ten animals each of 30 and 90 days chronicity.

Control animals

All chronic animal procedures were done unilaterely so that "experimental" and "control" sides of the animals could be compared clinicaly and electrophysiologicaly as described below. It was necessary

to have a control group not having chronic experimental lesions in order to determine the extent of variation between the two sides of a "normal" cat with no asymmetrical lesions. These animals were subjected only to the terminal experiment as described below.

Clinical evaluations

Clinical evaluation of neurological function in the animals was performed at the time of the terminal experiment and at varying intervals during the chronic period. The capacity for volitional movement and state of position sense was determined by observing gait and agility. Pain sense was tested by pin prick technique over all portions of the hind quarters. Knee jerk and Achilles tendon jerk were observed with respect to magnitude and symmetry. Muscle tonus in the hind limbs was observed and compared on the two sides of the animal. Flexion reflexes as evoked by noxious stimuli applied to various portions of the hind limbs was compared bilaterally with respect to magnitude and symmetry.

At the time of the terminal experiment the symmetry of the extensor rigidity produced by the decerebration procedure, the symmetry of the violent thrashing movements produced in the hind limbs by the acute cord transection procedure, and symmetry of the Schiff-Sherrington phenomenon in the forelimbs produced by the mid-thoracic transection also provided interesting clinical data.

Terminal experiment

The purpose of the terminal experiment was to make certain electrophysiological measurements as described below.

Preperation for the terminal experiment was as follows. The animals were anesthetized with ether. A tracheotomy was performed and the trachea cannulated. The common carotid arteries were tied and an external jugular

vein cannulated with PE 21 polyethylene tubing for administration of appropriate fluids and drugs. An opening two centimeters in diameter was made in the skull with rongeurs just cephalad to the bony tentorium cerebelli and lateral to the midline. The dural covering was opened and decerebration performed by sliding a small spatula along the tentorium toward the midline and ventrally. The adequacy of the decerebration could be gauged by the prompt onset of extensor rigidity which developed.

Ether was discontinued at this point. This allowed at least 90 minutes to elapse prior to the onset of recording. Occasional respiratory depression followed the decerebration. This was relieved by placing the animal on the respirator until spontaneous respirations resumed.

A small mid-thoracic laminectomy was performed and the entire cord transected by extradural pinch with jewelers forceps. In the chronicaly hemisected animals the acute transections were done about one centimeter below the hemisection site. The purpose of this procedure was to insure that descending input into the lumbar cord would be as symmetrical as possible. At least one hour was allowed to elapse between the transection procedure and the onset of recording.

The lumbosacral cord was exposed by dorsal laminectomy from the sacral hiatus to thelumbar one segment. The animal was immobilized with d-tubocurarine given intravenously in a dose such that skeletal muscle movement was just negated. Artificial respiration was initiated by means of a respirator adjusted to deliver a tidal volume and frequency of respiration judged by previous estimation of the animals spontaneous respiratory pattern.

The animal was then suspended on the experimental table by means of pins placed in the iliac crests, and a clamp attached to the T12 dorsal spinous process. The cutaneous and dorsal muscle tissues in the vicinity

of the laminectomy were formed into a pocket around the exposed cord in order to hold proper irrigation fluids (Krebs Ringer solution). The dura was then opened in the midline along the total length of the exposed cord and the edges sutured with 3-0 silk to the tissues lateral to the cord. The L7 roots were identified bilateraly by determining their point of exit through the intervertebral foramen, and also by virtue of their unique length and thickness relative to adjacent roots. Bilateral ventral and dorsal roots (L7) were then sectioned intraduraly just proximal to the dorsal root ganglion. The dorsal and ventral roots were then seperated and mobilised to their point of entrance into the cord. The cord and roots were then bathed in a solution of Krebs ringers solution the surface of which was covered with a thin layer of mineral oil.

The dorsal root was placed on a pair of bipolar stimulating electrodes of 20 gauge silver wire. Stimulation was provided by a Grass model S4 stimulator. A Grass model SIU 4 stimulus isolation unit was interposed between the stimulator and the stimulating electrodes. The stimulus waveform delivered to the animal was a squafe wave of 20 microsecond duration.

Bipolar recording electrodes of 20 gauge silver were employed on the proximal portions of both dorsal roots. Cord dorsum potentials were recorded by means of a silver ball electrode one mm in diameter. The indifferent electrode employed for cord dorsum potential recording was a one centimeter square silver plate attached to the exposed dorsal muscle mass lateral to the laminectomy. Cord dorsum potentials were led from a position one mm cephalad and one mm lateral to the center of the L7 dorsal rootlet entry into the cord.

All potentials were recorded on a Tektronix 502 RC coupled cathode ray oscilloscope employing push-pull input.

The following measurements were made bilaterely; dorsal root spike amplitude, dorsal root reflex amplitude and latency of onset, intramedullary spike onset latency and amplitude, Hla amplitude and peak latency, and Hlb amplitude and peak latency.

Stimulation frequency of one cycle per second was employed. Dorsal root spike and cord dorsum potentials were measured from threshold to maximum values. Dorsal root reflex measurements were made with a maximal stimulus (ie fully developed dorsal root spike).

Anatomical studies

Following the terminal experiment specimens were saved for histological evaluation. Chronicaly hemisected areas of the thoracic cord were sectioned and stained with H&E, Bodian silver, and Marchi stains to asses the extent of the hemisection. In chronicaly denervated and hemisected-denervated animals the sciatic nerves, dorsal root ganglion, and the L7 segment of the cord were sectioned and studied for evidence of chromatolysis or other degeneration of cell bodies or fibers. The sciatic nerves were studied for evidence of completeness of regeneration. Nisal, Bodian silver, and H&E stains were employed in the cord and ganglia studies. H&E and Bodian silver stains were used for studying sciatic nerve changes.

Treatment of Data

The electrophysiological measurements described above were treated as follows. In chronic animals ther experimental side values were divided by the control side values and multiplied x 100 to obtain the percent experimental values were of control values. The control animals were

treated in the same manner except that left side values were divided by right side values in 50% of the animals and vice versa in the remaining 50%. The means and standard deviations were computed for each group and the data analyzed statisticaly employing Student's t test.

Histological findings

At thirty days there was a mean incidence of 68% of cells in the ipsilateral L7 dorsal root ganglia of nerve crushed cats that displayed some degree of chromatolysis. Of the cells exhibiting chromatolysis 22% had type I changes (mild dissolution of the Nissl substance), 30% had type II changes (moderate to marked dissolution of Nissl substance) and 16% had type III changes (almost complete dissolution of Nissl substance and eccentricity of nucleus). In cats that had both hemisection of the spinal cord and nerve crush procedures the total incidence of chromatolysis in the dorsal root ganglion cells was 79% with 19% type I changes, 38% type II changes, and 22% type III changes. The mean percentage of cells in the dorsal root ganglia on the control side of the animals was 0.9%. The differences between the nerve crushed and the nerve crushed- cord hemisected animals was not significant. See figure I for photomicrographs of control and experimental dorsal root ganglia and ganglion neurones displaying the above discussed evidences of chromatolysis. Figure I also represents graphically the incidence of chromatolytic cell changes discussed above.

By 90 days the evidence of chromatolysis in dorsal root ganglion cells was seen in only 2.8% of cells counted in nerve crushed cats, and 3.3% in cord hemisected- nerve crushed cats. These values are not significantly different from control side counts. Statistical analysis of incidence of chromatolytic changes in the dorsal root ganglia of simply cord hemisected cats was not undertaken but preliminary counts indicated that the incidence was very similiar to control values.

Gross and histological examination of the Sciatic nerve at the nerve crush site was interesting in that even at 30 days the site of the original crush lesion was difficult to find. Microspopicaly small neurinoma like formations were occasionally seen

Figure I

A-F: Nissl stains of normal and chromatolyzed dorsal root ganglion cells

A: Section of ganglion from control animal. Note even dispersion of Nissl granules and concentricity of nucleŷ

B: Similiar section of ganglion from an animal that had had a nerve crush procedure 30 days prior. Note paucity of Nissl substance, perinuclear clumping of Nissl substance and examples of eccentricity of nucleus in many cells.

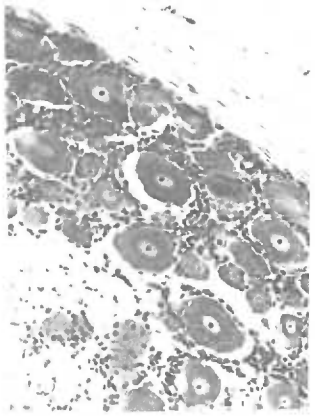
C: Normal appearing dorsal root ganglion cells from control side of denervated animal. The Nissl granules are clearly visible and may be seen to be quite evenly dispersed.

D: Mild chromatolytic changes in dorsal root ganglion cell from the experimental side of a 30 day cord hemisected-nerve crushed animal. The changes seen here of dissolution of Nissl substance, perinuclear clumping of the Nissl substance with a concentric nucleus are termed type I chromatolysis for convenience in this paper.

E: Type II chromatolytic changes in a dorsal root ganglion cell from the experimental side of a 30 day nerve crushed cat. More marked dissolution of the Nissl substance and mild eccentricity of the nucleus is apparent.

F: Type III chromatolytic changes in a dorsal root ganglion cell from a 30 day cord hemisected-nerve crushed animal. Note the marked eccentricity of the nucleus and marked dissolution of the Nissl substance.

G: Graphic representation of the percentage of cells in nerve crushed animals and in cord hemisected-nerve crushed animals of 30 days chronicity showing signs of chromatolysis. The total incidence is shown as well as the percentage of each of the various types of chromatolysis described above.



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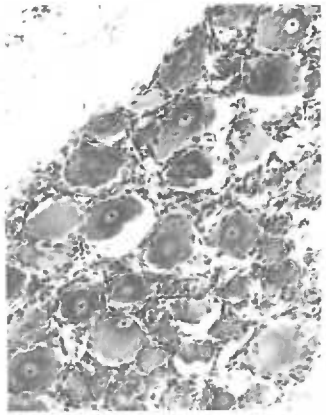

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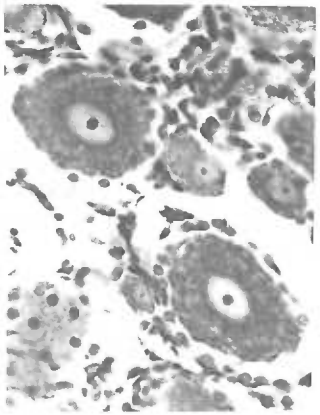

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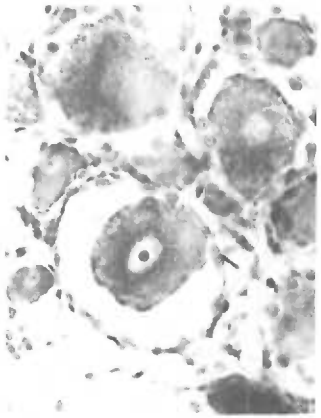

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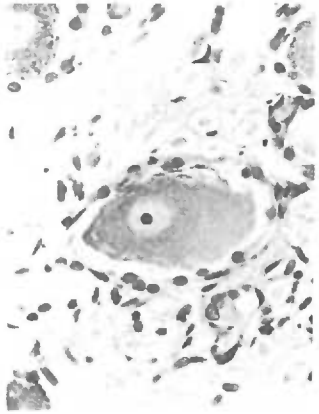
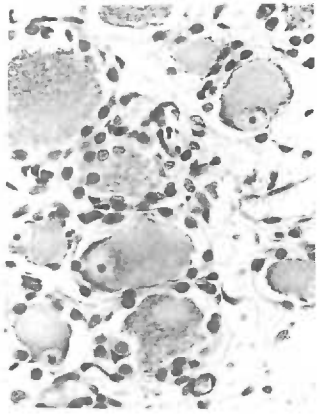
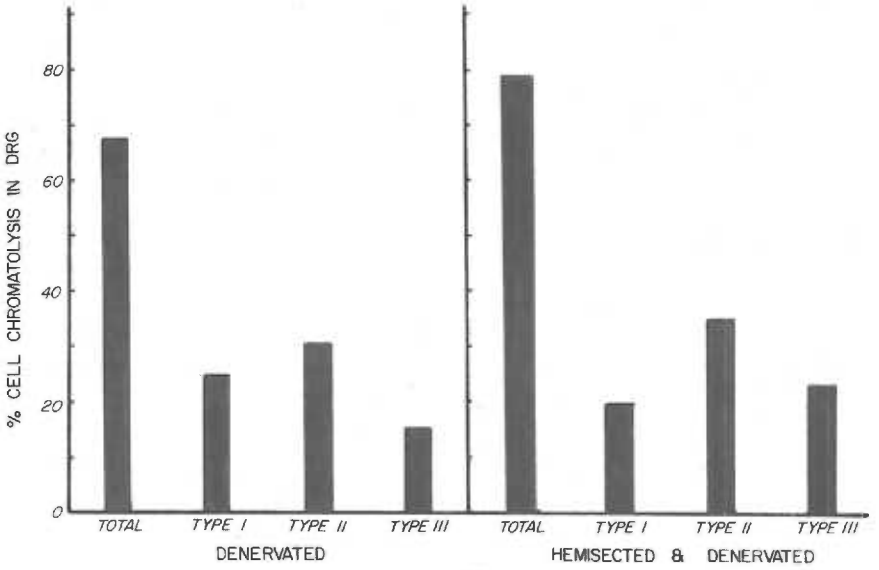


E



F


% CELL CHROMATOLYSIS IN DRG
0
20
40
60
80
TOTAL
TYPE I
TYPE II
TYPE III
DENERVATED
TOTAL
TYPE I
TYPE II
TYPE III
HEMISECTED & DENERVATED

G

Clinical Evaluations

Hemisected animals

Immediately following the hemisection of the spinal cord there was flaccidity in the ipsilateral hind limb. Within one hour however there was return of muscle tonus and reflexes. Flexor reflexes returned first and were stronger at one hour than were deep tendon reflexes. By 24 hours the muscle tonus was clearly stronger in the extensor muscles than in the flexors and the flexor reflex was lower on the ipsilateral side in threshold than on the control side. This increase in extensor tonus and decrease in flexor reflex threshold progressed somewhat for about 30 days. By thirty days the extensor tonus was quite marked and a resting clonus was often present. The flexor reflex had a low threshold and was very vigorous. Elicitation of the flexor reflex often resulted in a prolonged flexion of the extremity associated with clonus. Deep tendon reflexes elicited on the ipsilateral side also commonly gave rise to clonus whetehr or not a resting clonus was present.

Positive supporting reflexes were exaggerated as elicited by pressure on the pad or spreading of the otes in the ipsilateral hind limb, but hopping and placing reflexes were absent. Volitional use of the ipsilateral hind limb improved progressively over the 90 day period but was of startlingly good quality by thirty days. The animals could walk a narrow ledge forward and backwards although some mild error in tracking was usually present on the hemisected side.

Ninety day animals were clinically identical to the thirty day animals in the spinal cord hemisected group.

Following the mid collicular decerebration procedure carried out in preperation for the terminal experiment (see methods and materials) the

extensor rigidity was most marked in the ipsilateral forelimb. The hindlimbs were nearly equal, in extensor rigidity but sometimes the rigidity seemed greater in the ipsilateral hindlimb than in the contralateral hind limb.

When the spinal cord was acutely transected just caudal to the prior hemisection site the hindlimbs displayed vigorous thrashing movements that were markedly exaggerated in the hindlimb ipsilateral to the cord hemisection. Following subsidance of these movements the contralateral hind limb became flaccid while the ipsilateral hind limb tended to maintain increased extensor tonus, some clonus and a very active flexor reflex. Following the transection the forelimbs became equal with respect to the decerebrate extensor rigidity.

Nerve crushed animals

Following the nerve crush procedure the extremity was flaccid around the knee and ankle joints. There was no ebidence of sensation to about the junction of the proximal third and distal two thirds of the thigh. The animals tended to walk on the dorsum of their foot but interestingly failed to develop trophic ulcers as long as there was padding in the bottom of the cage. By thirty days there was some return of tonus around the knee joint and some evidence of feeling (albeit minimal) was noted at about knee level.

By ninety days there was equal strength to clinical examination in both hindlimbs including the foot. There was however a mild defecit in sensation around the foot as tested by pin prick. Stepping and placing reflexes were greater on the control side and the flexor reflex threshold was higher on the experimental side. Deep tendon reflexes were about equal in the two hind limbs.

It was of interest that most of the 90 day denervated animals

displayed a mild resting clonus and an increase in flexor tonus on the experimental side. Mild awkwardness with respect to volitional movement was apparent on the experimental side but the animals were very agile and could walk a narrow ledge both frontward and backward.

Following the midcollicular decerebration the extensor rigidity was more marked on the ipsilateral side in both the forelimbs and the hindlimbs.

During the acute transection of the cord the experimental side had very exaggerated movement compared to the control side. Following subsidence of these movements the hind limbs became quite symmetrical with respect to muscle tonus and reflex activity, ie the increased flexor tonus and clonus disappeared on the experimental side.

Hemisectdd and denervated animals

These animals were clinically identical with the denervated group at thirty days. By 90 days however the pattern was that of the hemisected group. The animals displayed increased extensor tonus, clonus and a very much lowered flexion reflex threshold. The flexion reflex was prolonged and accompanied by a marked clonus. Positive supporting reflexes were exaggerated as in the simply cord hemisected group. Hopping and placing reflexes were absent on the experimental side. Following decerebration the extensor rigidity was greatest in the ipsilateral forelimb and nearly equal in the hindlimbs.

During the acute cord transection procedure the vigorous thrashing movements were more intense on the experimental side. One hour following the transection procedure the experimental side had increased flexor tonus and a very hyperactive flexor reflex with clonus, and hyperactive deep tendon reflexes.

In summary the 30 day denervated and cord hemisected-denervated animals were characterized by findings of peripheral nerve defecit, both motor and sensory. The 30 and 90 day cord hemisected and the 90 day cord hemisected-denervated groups were very similiar clinically in that they featured the same pattern of clinical spasticity. The 90 day denervated animals displayed some characteristics of spasticity with increased flexor tonus and clonus. 90 day denervated animals with or without cord hemisection displayed nearly complete recovery from the peripheral nerve defecits noted at 30 days.

Electrophysiological findings

The below discussed findings are significant to the P= .05 level employing students t test unless otherwise specified. Tables I and 2 should be consulted for a summary of mean values, standard deviations, and significance levels. Figure2is referred to for some representative oscillograph tracings of the discussed findings.

Cord hemisected cats

Significant findings include an increase in the peak amplitude values of the N1a component of the cord dorsum potential on the hemisected side as compared to the control side. The cord hemisected side values were 27.8% higher than the control values at 30 days, and 28.1% higher at 90 days. The N1b peak amplitude values were also increased over control values by 21.9% at 30 days and 20.6% at 90 days. There was no significant changes in the values of the IMS or DRS in the experimental side as compared to the control side. Likewise there were not any significant differences in the latency of peak N1b values or in the onset latency of the DRR. The amplitude of the DRR was not significantly altered.

At threshold stimulation there was no difference in the ratio of amplitude of N1b to N1a as compared to control animals, but at maximum stimulation there was an increase of about 10% in this ratio as compared to control animals in both 30 and 90 day cats (P=.10). See figures 3, 4, and 5 for graphic representation of the above described findings.

Denervated cats

At 30 days the records displayed the following amplitude depressions: DRS 34.4%, IMS 30.8%, N1a 62.7%, N1b 37.2% and DRR 75.7%. The peak latency of N1b was increased 40.5%. The ratio of N1a to N1b was not altered compared to controls at either submaximal or maximal stimulation. See figures 3, 4, and 5.

The 90 day animals displayed the following amplitude depressions: DRS 17.9%, IMS 16.2%, N1a 43.8%, N1b 11.1% (P=.10) and DRR 31.1%. At 90 days there was no significant increase in either N1b peak latency, or DRR onset latency. As at thirty days there was no difference in the N1b/N1a ratio. See figures 3,4,and 5.

Significant differences between the 30 day and 90 day denervated cats included a recovery of the DRS amplitude of 16.5% (P=.10), a recovery of the IMS amplitude of 14.6% and a 18.9% recovery of the N1a amplitude. The nearly complete recovery of the latencies of N1b and the DRR were both significant to the .01 level. The DRR amplitude recovered by 44.6%.

Cord hemisected and denervated cats

At thirty days the following amplitude depressions were noted: DRS 40.6%, IMS 36.8%, N1a 61.4%, N1b 48.8% and DRR 78.7%. The peak latency of N1b was increased 24.2% and the onset latency of the DRR 31.6%.

With maximum stimulation the ratio of N1b to N1a was decreased by 16% (P=.10). See figures 3,4, and 5.

At 90 days the following amplitude depressions were noted: DRS 22.2%, IMS 18.8%, N1a 28.5%, N1b 10.2% (P=.10) and DRR 33.2%. The N1b peak latency and DRR onset latency were no longer increased. See figures 3,4, and 5.

Significant differences between the 30 and 90 day animals included recovery of the DRS amplitude by 18.4% (P=.10), recovery of the IMS amplitude by 1[illegible].0%, recovery of the N1a amplitude by 32.9% and recovery of the DRR by 45.5%. As noted above the previously increased latencies of the N1b and DRR at 30 days had returned to normal by 90 days.

Significant differences between the denervated and the cord hemisected denervated series was limited to a significantly more profound depression of the N1a component of the cord potential at 90 days in the latter series.

Figure 2

Positive print of tracings taken on kymograph directly from oscilloscope. All tracings taken at maximum stimulus

A- Cord hemisected cats
1- Cord dorsum potentials from control side of a 30 day cord hemisected cat. See A-3 for labeling of cord potential components.
2- Same cat, hemisected side of cord.
3- Cord dorsum potential from control side of a 90 day cord hemisected cat.
4- Same cat, hemisected side of cord.

B- 30 day cord hemisected-denervated cat.
1- Dorsal root spike of control side.
2- Cord dorsum potential of control side.
3- Dorsal root spike of experimental side.
4- Cord dorsum potenti al of experimental side.

C- 30 day denervated cat
1- High gain tracing of dorsal root spike on control side showing dorsal root reflex.
2- Cord dorsum potential of control side.
3- Same as 1 above except experimental side.
4- Same as 2 above except experimental side.

D- 30 day cord hemisected- denervated cat.
1- High gain tracing of dorsal root spike on control side of animal showing dorsal root reflex.
2- Cord dorsum potential of control side.
3- Same as 1 above except experimental side.
4- Same as 2 above except experimental side.

E- 90 day cord hemisected- denervated cat
1- High gain tracing of dorsal root spike on control side of animal showing dorsal root reflex.
2- Cord dorsum potential of control side.
3- Same as 1 above except experimental side.
4- Same as 2 above except experimental side.

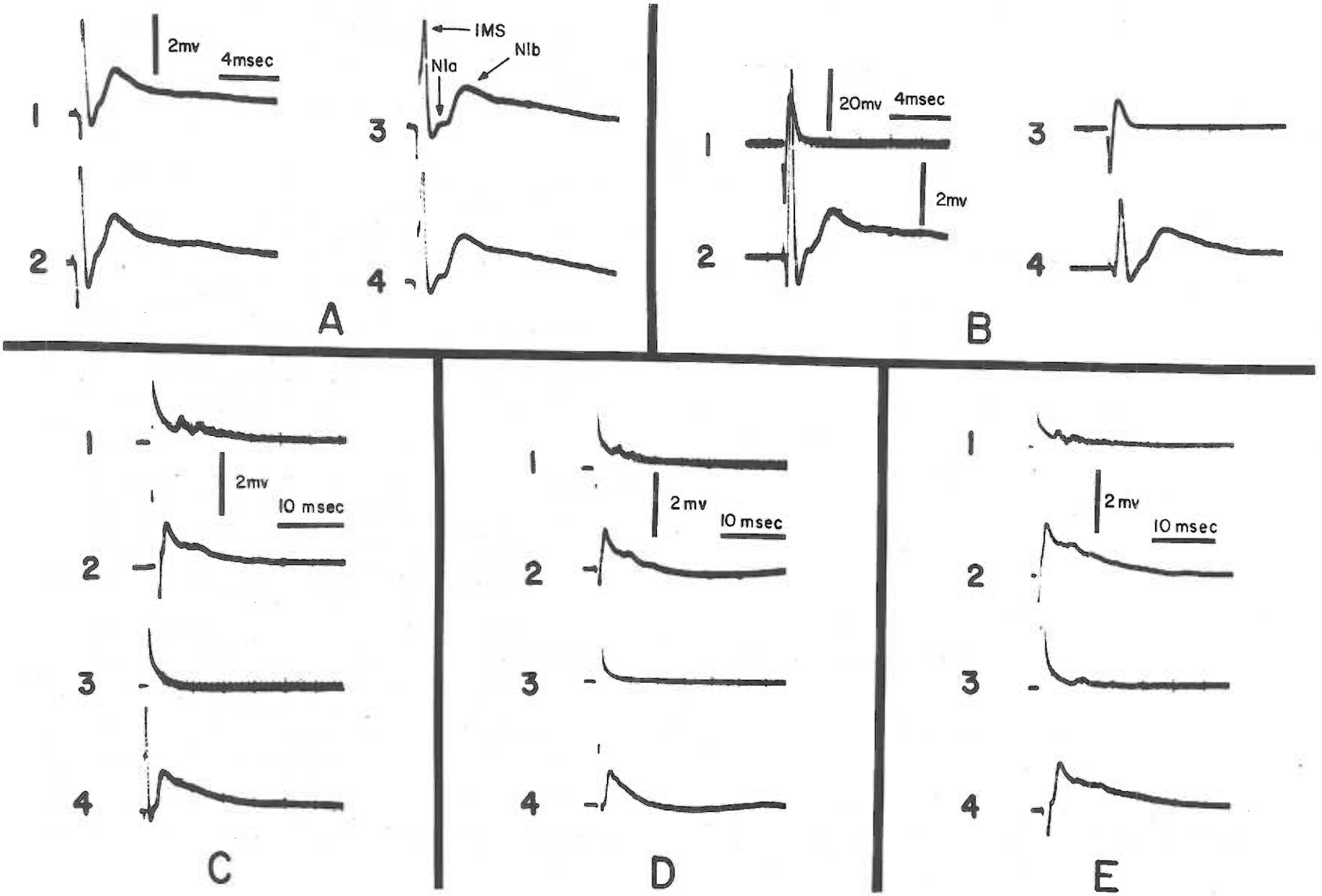

2mv
4msec
1
2
IMS
NIa
NIb
3
4
A
20mv
4msec
2mv
1
2
3
4
B
2mv
10 msec
1
2
3
4
C
2 mv
10 msec
1
2
3
4
D
2 mv
10 msec
1
2
3
4
E

Figure 3

Graphic illustration of the maximum peak amplitude values of the dorsal root spike (DRS), intramedullary spike (IMS), N1a component of the cord potential, and N1b component of the cord potential.

Abscissa represents percent experimental value is of control side value. Each point represents mean value of ten animals.

Ordinate represents days after experimental lesions were made (30 days or 90 days).

Each line represents one of the four basic series of animals:
Hemisected stands for cord hemisected animals.
Denervated stands for peripheral nerve crushed animals.
Hemisected and denervated stands for cord hemisected and peripheral nerve crushed animals.
Control stands for animals intact except for the terminal experiment.

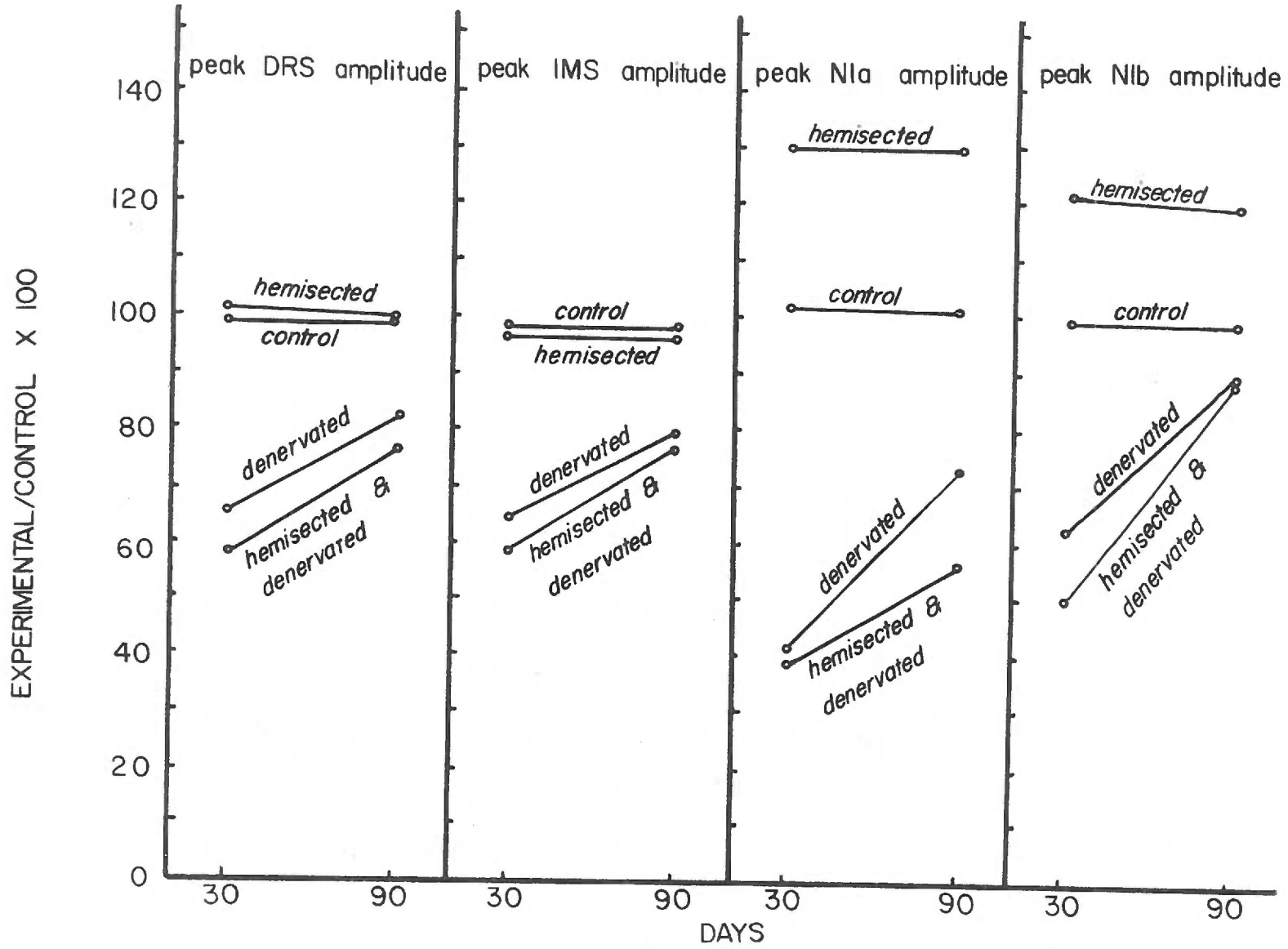

peak DRS amplitude
peak IMS amplitude
peak NIa amplitude
peak NIb amplitude
EXPERIMENTAL/CONTROL X 100
140
120
100
80
60
40
20
0
30
90
DAYS
hemisected
control
denervated
hemisected &
denervated

Figure 4

Graphic illustration of N1a peak latency, N1b peak latency, dorsal root reflex (DRR) peak amplitude, and dorsal root reflex (DRR) onset latency. All values measured at maximum stimulus.

Abscissa represents percent experimental value is of control side value. Each point represents mean value of ten cats.

Ordinate represents days after experimental lesions were made (30 days or 90 days).

Each line represents one of the four basic series of animals:
Labeling of lines is as in figure 3.

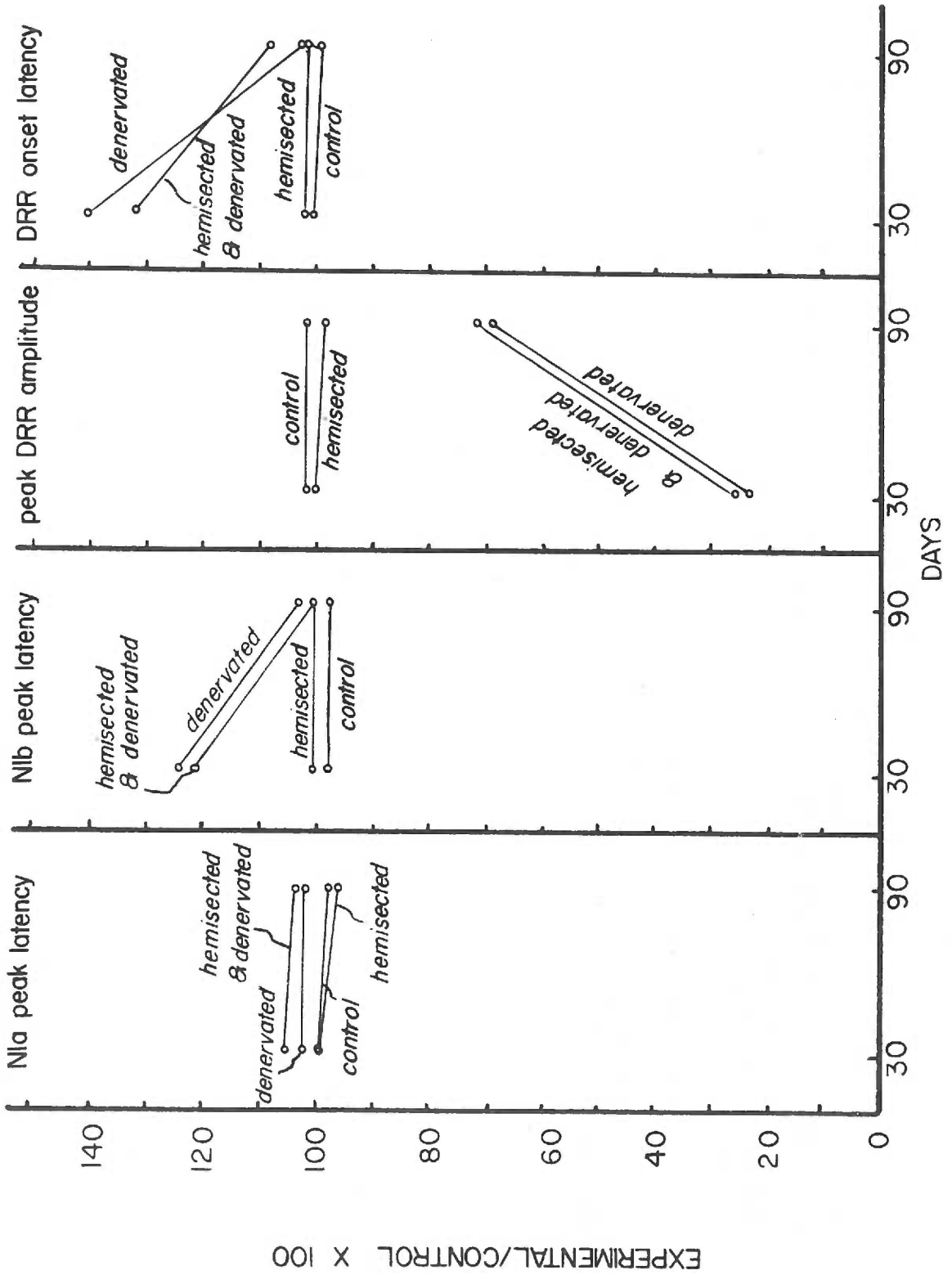

N1a peak latency
N1b peak latency
peak DRR amplitude
DRR onset latency
hemisected & denervated
denervated
hemisected
control
EXPERIMENTAL/CONTROL X 100
DAYS
0
20
40
60
80
100
120
140
30
90

Figure 5

Graphic illustration of relationship of presynaptic (N1a) and post synaptic (N1b) components of cord dorsum potential with threshold to maximum stimulus.

Abscissa and ordinate represent experimental side value at a given stimulus strength divided by control side maximum values. This is multiplied by 100 to give a percent value.

Labeling of each of the four basic series of cats is as in figure 3.

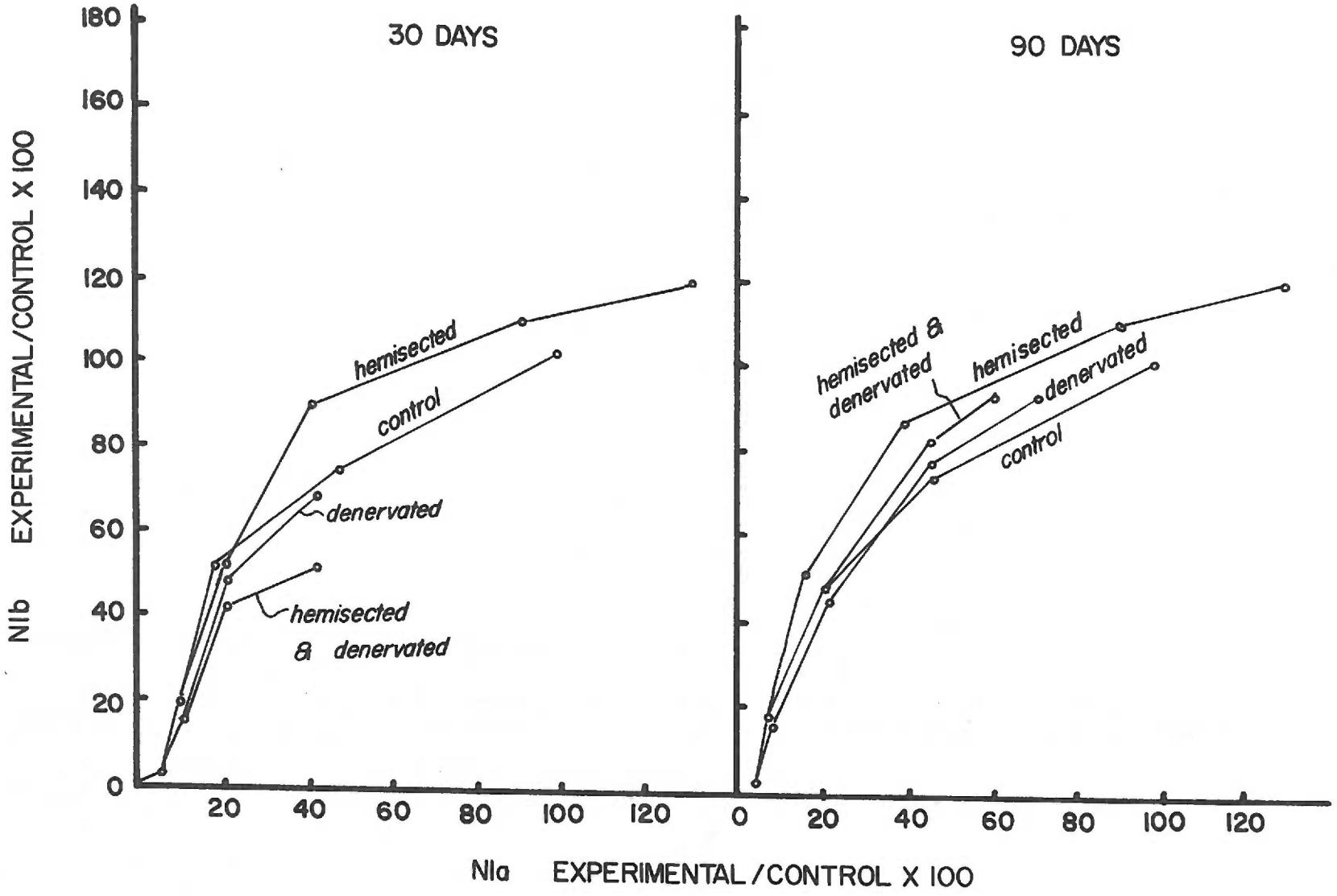

30 DAYS
90 DAYS
NIb EXPERIMENTAL/CONTROL X 100
NIa EXPERIMENTAL/CONTROL X 100
0
20
40
60
80
100
120
140
160
180
hemisected
control
denervated
hemisected & denervated
hemisected & denervated
hemisected
denervated
control

TABLE I

Amplitude

Null Hypothesis	Control mean*	Exp. mean*	s Control	s Exp.	t	p
Control DRS = 30 day denervated DRS	99.1	64.7	6.5	8.5	10.1144	.01
Control DRS = 90 day denervated DRS	99.1	81.2	6.5	8.0	5.4242	.01
Control DRS = 30 day hemisected & denervated DRS	99.1	58.5	6.5	6.8	13.0968	.01
Control DRS = 90 day hemisected and denervated DRS	99.1	76.9	6.5	7.6	3.9355	.01
30 day denervated DRS= 90 day denervated DRS					1.7715	.10
Control IMS = 30 day denervated IMS	99.3	64.5	5.7	7.9	4.7331	.01
Control IMS = 90 day denervated IMS	99.3	79.1	5.7	10.9	2.1041	.05
Control IMS = 30 day hemisected and denervated IMS	99.3	58.5	5.1	12.4	4.8081	.01
Control IMS = 90 day hemisected and denervated IMS	99.3	76.5	5.7	11.4	2.5731	.05
30 day denervated IMS = 90 day denervated IMS					5.6162	.01
30 day hemisected & denervated IMS = 90 day hemisevted & denervated IMS					2.5711	.05
Control Nla = 30 day hemisected Nla	101.5	129.4	10.1	19.0	2.4438	.05
Control Nla = 90 day hemisected Nla	101.5	129.6	11.2	18.3	2.3416	.05

AMPLITUDE

Null Hypothesis	Control mean*	Exp. mean*	s Control	s Exp.	t	p
Control N1a= 30 day denervated N1a	101.5	38.8	10.1	16.7	10.1138	.01
Control N1a = 90 day denervated N1a	101.5	57.7	10.1	15.7	4.8666	.01
Control N1a = 30 day hemisected and denervated N1a	101.5	40.1	10.1	10.7	10.9633	.01
Control N1a = 90 day hemisected and denervated N1a	101.5	73.0	10.1	16.7	3.5900	.01
30 day denervated N1a = 90 day denervated N1a					3.5454	.01
30 day hemisected & denervated N1a = 90 day hemisected & denervated N1a					3.3092	.01
90 day denervated N1a = 90 day hemisected & denervated N1a					2.9000	.01
Control N1b = 30 day hemisected N1b	99.5	121.4	8.3	9.3	2.4333	.05
Control N1b = 90 day hemisected N1b	99.5	120.1	8.3	13.9	3.8418	.01
Control N1b = 30 day denervated N1b	99.5	62.3	8.3	15.8	5.3148	.01
Control N1b = 90 day denervated N1b	99.5	88.4	8.3	10.02	1.7619	.10
Control N1b = 30 day hemisected and denervated N1b	99.5	50.7	8.3	11.1	8.1333	.01
Control N1b = 90 day hemisected and denervated N1b	99.5	89.3	8.3	9.5	1.7560	.10

AMPLITUDE

Null Hypothesis	Control mean*	Exp. mean*	& Control	Exp.	t	p
30 day denervated N1b = 90 day denervated N1b					4.0154	.01
30 day hemisected & denervated N1b = 90 day hemisected & denervated N1b					6.6551	.01
Control DRR = 30 day denervated DRR	102.7	27.0	30.7	17.9	6.3083	.01
Control DRR = 90 day denervated DRR	102.7	71.6	30.7	22.1	2.2214	.05
Control DRR = 30 day hemisected and denervated DRR	102.7	24.0	30.7	17.6	6.5583	.01
Control DRR = 90 day hemisected & denervated DRR	102.7	69.5	30.7	16.9	3.0182	.01
30 day denervated DRR = 90 day denervated DRR					4.6947	.01
30 day hemisected & denervated DRR = 90 day hemisected & denervated DRR					5.5487	.01

* Note: Means represent experimental value divided by control value x 100 in the experimental animals. Control means are values obtained from one side of animal divided by value from other side of animal (50% left side/ right side, 50% right side/ left side) x 100.

Table 2

LATENCY

Null Hypothesis	Control mean*	Exp. mean*	s Control	s Exp.	t	p
Control N1b = 30 day denervated N1b	99.4	125.8	9.6	14.3	2.1990	.05
Control N1b = 30 day hemisected and denervated N1b	99.4	123.6	9.6	11.0	2.2441	.05
Control DRR = 30 day denervated DRR	101.5	142.0	7.6	17.2	2.2777	.05
Control DRR = 30 day hemisected & denervated DRR	101.5	133.1	7.6	14.8	2.2333	.05

* Note: Means represent experimental value divided by control value x 100 in the experimental animals. Control means are values obtained from one side of animal divided by value from other side of animal (50% leftside/ right side, 50% right side/ left side) x 100

Discussion

The purpose of this study was to determine some of the effects of peripheral nerve crush lesions on spasticity induced by hemisection of the spinal cord. Evaluations were made as to the clinical effect of the nerve crush procedure on the pattern of spasticity, and on the electrophysiological measurements of the dorsal root spike, cord potential, and the dorsal root reflex. In particular this study was concerned with the effects of the nerve crush procedure on the sprouting of dorsal root afferent fibers into the cord following cord hemisection. This sprouting phenomenon has previously been correlated with an increase in the N1a component of the cord potential which has been ascribed to the action potential of the dorsal root afferent terminals (92). Previous work has indicated that the increase in the amplitude of the N1a component of the cord potential following cord hemisection correlates with the histological demonstration of increased numbers of afferent terminals secondary to their sprouting following cord hemisection (18). As previously stated (see statement of problem) the hypothesis to be tested by this study was that the peripheral nerve crushing simultaneous with hemisection of the spinal cord would decrease the capacity of the dorsal root afferent fibers to sprout onto spinal neurones partially denervated by the hemisection procedure. This should result in a decreased N1a potential relative to the increase previously described subsequent to simple cord hemisection. Further if the sprouted afferent terminals are directly related to the spasticity induced by cord hemisection the pattern of spasticity should be altered or abated if the N1a potential is depressed by the nerve crush procedure.

The finding of a depressed N1a potential in the animals that were hemisected and denervated fails to satisfy this hypothesis since the

pattern of clinical spasticity remained essentialy unchanged. In this study the finding of depression of the N1a potential secondary to the denervation procedure was complicated by the findings of a) electrophysiological depression of all presynaptic elements studied and a high indidence of chromatolytic changes in the dorsal root ganglion, and b) more profound depression of the N1a potential in animals that were hemisected and denervated as compared to the simply denervated animals.

The finding of a high incidence of chromatolytic changes in the dorsal root ganglia cells 30 days following the denervation procedure suggests that the effect of this procedure on the afferent limb of spinal reflex activity may be due to injury (111) rather than disuse (117) of the presynaptic pathways. Some authors have considered that the phenomenon of retrograde chromatolysis is due to injury secondary to bleeding of cytoplasm from the injured axonal stump, and a relative deficiency of cytoplasm as the process of peripheral renegration occures (111)(112). It is of interest to consider that the chromatolytic process itself could be due to disuse of the neurones involved since following the peripheral nerve lesion the neurone is separated from its normal source of excitation.

In this study the peripheral nerve was allowed to regenerate. Regeneration was quite complete from the standpoint of clinical examination by 90 days. Corresponding with this was a statistically complete recovery of the ganglion cells with respect to evidence of chromatolytic changes. However the electrophysiological measurements of the dorsal root spike and presynaptic components of the cord potential suggests a permanent defecit in the afferent limb of spinal pathways, possibly reflecting a certain incidence of cell death in the dorsal root ganglion. This would seem

to be most logically explained on the basis of cell injury, especially since the study of chemical changes in regenerating neurones also seems to support strongly an injury basis for chromatolysis(112).

The depression of the dorsal root spike and presynaptic components of the cord potential could be explained on either the basis of a smaller spike amplitude per active fiber, or a decrease in the total number of fibers excited by the stimulus. The latter possibility seems most likely since work by Eccles et al(118) has indicated that the spike height is unchanged in motoneurones undergoing the process of retrograde chromatolysis.

It was found in this study that the N1a component of the cord potential was more profoundly depressed at 30 days in the denervated animals (with or without cord hemisection) than the other presynaptic elements measured (IRS and IMS). This can partially be attributed to the corresponding depression of the N1b component of the cord potential since N1a is a contaminated measurement in that it occures on the ascending limb of the N1b deflection. This is not a complete explanation however. The significantly more profound depression of N1a in the 90 day hemisected as compared to the simply denervated group cannot be explained on this basis since N1b had recovered to nearly equal values in the two groups. The more profound depression of N1a in the hemisected and denervated group as compared to the simply denervated group cannot be explained on the basis of greater injury to the parent fiber by the hemisection procedure since the incidence of chromatolysis in the dorsal root ganglion cells was not significantly different between the two groups. Also other presynaptic elements were not significantly different at 90 days between these two groups.

The more profound depression of the afferent terminal potential (N1a)

in the hemisected and denervated group might be explained by a regression or withdrawal of some of the afferent terminals from positions they normally occupy on spinal neurones. If however the terminals could not regain their prior positions(in this group in contrast to the simply denervated group) the more profound residual depression of N1a might be explained on the basis that the terminals had regressed permanently. A plausible reason for the terminals not being able to regain their prior positions would be that the positions were occupied in the interim by terminals from other sources, ie interneuronal, descending or crossed fibers.

It is puzzling that N1b post synaptic potentials returned to nearly normal and equal levels with respect to both amplitude and latency in the two groups despite the greater afferent terminal depression in the hemisected-denervated group. It must be pointed out however that it has not been established that the N1a afferent terminals are causally related to the N1b potentials. Indeed some evidence has been presented to suggest that the terminals represented by the N1a potentials may be concerned with other post synaptic elements (107)(108)(109). It is also possible that the terminals that remain on the N1b interneurones might keep the post synaptic elements in a sufficient state of tonus such that stimulation of the afferent terminals that did retain their positions might be able to elicit a near normal response. Chemical sensitization of the N1b post synaptic elements to impulses from remaining intact afferent terminals represents another possibility.

The nearly identical pattern of clinical spasticity in the cord hemisected as compared to the cord hemisected-denervated group indicates that the spasticity produced by hemisection of the spinal cord does not correlate with the absolute magnitude of the N1a component of the cord potential. This is not evidence against the sprouting of afferent

terminals as a cause of spasticity however since the pattern of sprouting might be of much greater significance than the quantity of sprouting. That sprouting with respect to afferent terminals seems to occur on a pattern basis rather than a random basis has recently been demonstrated by Eccles et al (94)(95).

It is interesting that the simply denervated animals displayed some of the signs of spasticity at 90 days, ie clonus, increased flexor tonus, and a very exaggerated movement response in the ipsilateral hind limb when the cord was acutely transected. In the preceeding paragraphs an hypothesis has been presented that sprouting of descending, interneuronal, or crossed fibers may occur onto positions vacated by injured afferent fibers; this could also represent a plausible explanation for the signs of spasticity demonstrated by the 90 day denervated animals. Alternate explanations include chemical sensitization of the involved post synaptic elements, and a possible relation of this evidence of spasticity to the finding of a significantly depressed dorsal root reflex. If Eccles et al (124) are correct in their hypothesis that the DRR is related to pre-synaptic inhibition, then depression of this reflex might be equivalent to disinhibition of the incoming impulses on the fibers involved. The increased flexor tonus is especially interesting in this respect since the flexor muscles seem to have a relatively major share of the DRR(123).

In this study the increase in both N1a and N1b components of cord potential following hemisection of the spinal cord has been confirmed. The latencies of post synaptic elements studied were unchanged from control values. There was no significant difference found in the DRR between the cord hemisected and control animals.

SUMMARY

1- This study was based on the hypothesis that peripheral nerve crush lesions should decrease the sprouting of afferent terminals onto positions vacated on spinal neurones by hemisection of the spinal cord. This should result in a relative decrease in that component of the cord potential related to afferent terminals (N1a) and abate or modify the clinical pattern of spasticity as compared to animals having simply a cord hemisection procedure.

2- It was found that the N1a potential was significantly depressed in animals having both nerve crush and cord hemisection procedures. However the clinical pattern of spasticity was not abated or significantly altered. This finding was complicated by the findings of depression of all presynaptic components, and by the finding that the N1a potential was more depressed in the animals that were cord hemisected and denervated as compared to the simply denervated animals.

3- It is concluded that this study did not demonstrate the role played by the sprouting of dorsal root afferents in spasticity induced by hemisection of the spinal cord sonce it did not discriminate between the importance of the pattern of sprouting as compared to the quantity of sprouting as indicated by the absolute magnitude of the N1a potential. However it may be concluded that the clinical pattern of spasticity is not related to the absolute magnitude of the N1a potential.

4- It is concluded that peripheral nerve section such as done in this study cannot justifiably be used to evaluate the role of disuse of afferent fibers on spinal cord pathways since a high incidence of retrograde chromatolysis was demonstrated secondary to the afferent fiber lesion. The etiology of the phenomenon of retrograde chromatolysis is discussed and it is concluded that it is a reflection of injury

to the parent neurone.

5- Reasons for the finding of more profound residual depression of the N1a component of cord potential in the cord hemisected-denervated animals as compared to the simply denervated animals are discussed.

6- The increase in the N1a and N1b components of the cord potential following cord hemisection is confirmed. No change in latency of the post synaptic component was noted. No significant variations from control values are noted in the dorsal root reflex following cord hemisection.

7- Findings of increased flexor tonus, clonus, and exaggerated ipsilateral hind limb movement during acute cord transection are described in the 90 day denervated animals. It is pointed out that this is correlated with the finding of significant depression of the dorsal root reflex in this group of animals, but not necessarily causally related.

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