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Title of Review: Axonal Regeneration in Mammals with Spinal Cord Injury

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ABSTRACT

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Benjamin L. Wong, Master of Science, 1983

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Since Ramon y Cajal's classic demonstration of abortive regeneration in injured mammalian spinal cord, numerous studies have sought to identify conditions or to develop therapeutic methods that are capable of maintaining continual axonal regeneration in injured neural tissue. This review examines some of the major developments in the field of central nervous system (CNS) regeneration research. These developments have revealed important aspects regarding the histology and physiology of traumatized spinal cord. A growing area of spinal cord injury research lies in identifying the factors related to neuronal plasticity and axonal regeneration of the spinal cord. This review will discuss those factors that are considered responsible for inhibiting axonal regeneration in the traumatized mammalian spinal cord. In addition, this review also discusses some of the experimental approaches to the enhancement of axonal regeneration after spinal cord injury. Future avenues for research in CNS regeneration are suggested in the final part of this review.

Axonal Regeneration in Mammals with Spinal Cord Injury

by

Benjamin Lee Wong

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To Sophia

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TABLE OF CONTENTS

I. INTRODUCTION

Since ancient times, it has been recognized that the integrity of the spinal cord is essential for voluntary movement, and that injury to the spinal cord can result in paralysis of the afflicted individual. Today we are becoming increasingly aware of the vulnerability of the spinal cord. In the United States alone, more than 200,000 persons, most of them young, are paralyzed as a result of damage to their spinal cords (Marx, 1980). The annual incidence for traumatic damage of the spinal cord ranges regionally from 1.3 to 3.3 per 100,000 population and has been increasing steadily (Kurtzke, 1977).

The earliest descriptions of human paraplegia and quadriplegia are contained in the Edwin-Smith Surgical Papyrus (Breasted, 1930). Egyptian physicians clearly described the effects of traumatic damages of the neck nearly 5,000 years ago (Windle, 1980). Symptoms of spinal patients were clearly described, instructions for examining the patients were given, and a dire prognosis was indicated. Ancient methods of treating the incapacitated patients were futile and often resulted in further trauma to the individuals (Benes, 1968).

Attempts to investigate spinal cord injury using animal models are unknown prior to Galen's time (130-200 A.D.). It is doubtful if the medical authority before Galen's time even considered the possibility of spinal cord regeneration (Jewett and McCarroll, 1980). Galen's experiments with young monkeys and some domesticated animals is a first in the history of experimental neurology (Prendergast, 1930). When Galen exposed the spinal cord of an animal and made a longitudinal incision along the entire length of its spinal cord, the animal continued to

breathe and exhibit limb movements. When the spinal cord of the same animal was transected, however, paralysis was observed below the level of the transection. Respiratory function was spared when the transection was introduced at the lower cervical region. Even though Galen described medicine to treat animals with nerve wounds, it is not clear whether he used sutures or believed that spinal cord axons could regenerate (Jewett and McCarroll, 1980). After Galen's death, there was a hiatus of more than 1,600 years before experiments on spinal cord were resumed (Benes, 1968).

In 1848, Brown-Sequard made a dramatic advance in the technical aspect of spinal cord research by combining histological and physiological methods to investigate spinal cord injury (Windle, 1980). He transected the upper thoracic spinal cord of adult pigeons and noted the results. Complete recovery of sensory and motor functions was claimed in one pigeon 14 months postoperatively. It was also reported that nerve fibers extended into the scar tissue at the site of the transection. These findings are dubious, however, because the histological techniques used were poor (Puchala and Windle, 1977). Other experiments (Sgobbo, 1890; Foster, 1911) involving short-term studies on spinal trauma in birds failed to support the phenomenon of functional regeneration in the CNS.

In 1894, Stroebe described the presence of regenerating nerve fibers in the scar tissue between the ends of severed spinal cord in rabbits. The regenerating fibers did not, however, re-enter either end of the transected spinal cord. Several investigators (Bielschowsky, 1909; Ssamarin, 1926; Ramon y Cajal, 1928) corroborated Stroebe's observations with the aid of silver staining methods.

The foundations of our understanding of nerve regeneration were established by Ramon y Cajal at the beginning of the century. Based on studies originally published in 1906, 1910, and 1911, he (1928) described in detail the process of degeneration and regeneration of spinal axons after partial or complete transection of the lumbar spinal cord in kittens and puppies. Three or 4 days after the experimental manipulation, some fibers of small to moderate diameter began to regenerate. The ends of the transected axons exhibited "growth cones" similar to those observed in regenerating peripheral axons. Growth of the transected axons was aborted, however, within 2 weeks of the operation; consequently, restitution of function did not occur. Ramon y Cajal (1928) did not consider this inability of the injured spinal cord to regenerate to be an intrinsic characteristic of the CNS; rather, he considered it to be the result of the lack of a favorable environment in the scar tissue. Subsequent studies (Sugar and Gerard, 1940; Windle, 1956; Campbell, et. al., 1957, 1973; Freeman, 1962; Kao, et. al., 1977a; Naftchi, et. al., 1980; Reier, et. al., 1982) have confirmed Ramon y Cajal's observations that the regenerative process of severed spinal axons became abortive within 2 weeks of transection.

Currently there are no effective means, either chemically or physically, for restoring sensorimotor functions lost as a result of trauma to the spinal cord. Attempts to induce axonal regeneration and reestablish neural connections have met with frustration and failure. Nevertheless, in spite of the lack of success, our understanding of the reaction of the CNS to injury and its capacity to regenerate has improved dramatically in the past 20 years. Meticulous studies have demonstrated that neurons in the CNS are intrinsically capable of

regenerating, although they normally do not succeed in establishing functional connections (Windle, 1956; David and Aguayo, 1981; Guth, et. al., 1981; Benfey and Aguayo, 1982). Current experimental work seeks to identify the elements responsible for obstructing the regenerative process in the CNS, the conditions conducive to nerve regeneration, and the factors that may enhance the regenerative capacity of intrinsic neurons.

II. ANATOMICAL AND PHYSIOLOGICAL CHANGES

A. Clinical Correlations

When the spinal cord is transected completely, several disorders are immediately observed below the level of the lesion: (1) cessation of sensory (somatic and visceral) function, (2) loss of motor function, and (3) disappearance of reflex activity. The paralysis below the level of the lesion is of the flaccid type. Nearly always there is paralysis of bladder and bowel functions due to the interruption of descending autonomic fibers which control these functions. Defecation occurs involuntarily, however, after long intervals (Carpenter and Sutin, 1983). Sweat secretion is abolished below the level of the lesion, and the blood pressure decreases temporarily (Brodal, 1981). Sexual functions are also impaired in the male.

This state of complete loss of neural function in the spinal cord caudal to the lesion has been termed "spinal shock," or diaschisis. The cause of spinal shock has not been identified, but is presumed to be the result of the abrupt cessation of tonic stimulation of spinal neurons by excitatory impulses in the descending pathways (Ganong, 1979; Brodal, 1981; Carpenter and Sutin, 1983). In primates and humans, disruption of the corticospinal tract is believed to be related to the onset of spinal shock (Chusid, 1982). The phenomenon of spinal shock is transient; and it is generally agreed that the period of spinal shock is proportionate to the degree of encephalization of motor function in various species (Sherrington, 1898; Fulton and McCouch, 1937; Ganong, 1979). In man this period ranges from 1 to 6 weeks and averages about 3 weeks (Carpenter and Sutin, 1983).

The recovery of reflex excitability signifies the end of the period of spinal shock. The spinal cord below the lesion regains some of its functions, although it lacks supraspinal input (Brodal, 1981). This functional recovery in the isolated spinal segment has been classified into 4 phases (Kuhn, 1950): (1) minimal reflex activity (3 to 6 weeks), (2) flexor spasms (6 to 16 weeks), (3) alternate flexor and extensor spasms (after 4 months), and (4) predominant extensor spasms (after 6 months). In the first phase, signs of reflex activity of the muscles may be detected, although the deep tendon reflexes remain absent. One prominent characteristic of this phase is the Babinski sign, which may be elicited bilaterally. The flexor muscles in the extremities may contract slightly in response to nociceptive stimuli. This flexor muscle response begins distally and progressively involves proximal muscle groups (Carpenter and Sutin, 1983). During this phase the muscles remain flaccid. The detrusor muscle of the bladder, however, gradually hypertrophies, and intermittent dribbling of urine occurs as the muscle briefly overcomes the resistance of the external sphincter (Carpenter and Sutin, 1983). Eventually, continual hypertrophy of the bladder wall leads to occasional spontaneous emptying of the bladder in response to moderate filling. Concomitantly, automatic reflex activity of the rectum also reappears, and sweat secretion is possible below the level of the lesion (Brodal, 1981).

In the phase of flexor muscle spasms, the muscles resume some of their tone and respond more intensely to nociceptive stimuli. This phase is also characterized by Sherrington's triple flexion response (Brodal, 1981), which may be elicited by relatively mild nociceptive stimuli and which involves flexion of the lower extremities at the hip,

knee, and ankle. The exaggerated form of this response is referred to as the mass reflex, in which a mild stimulus results in powerful bilateral triple flexion response, often accompanied by massive abdominal contractions, emptying of the bladder and rectum, sweating, pallor, and blood pressure fluctuations (Ganong, 1979; Luckmann and Sorensen, 1980). Although distressing to the patient, the mass reflex may, however, be used to give the patients some degree of bladder and bowel control. They may be trained to initiate urination and defecation by stroking or pinching their thighs, thereby eliciting a mild mass reflex (Luckmann and Sorensen, 1980; Chusid, 1982). As the extensor muscle tone begins to increase, 4 months after spinal cord transection, the intensity of the mass reflex decreases (Adams, 1973; Carpenter and Sutin, 1983). At this stage there are alternating flexor and extensor spasms, and the fixed position may be either extension or flexion of the knees and hips (Rowland, 1981), but within a relatively short time extensor muscle tone predominates (Adams, 1973; Carpenter and Sutin, 1983). Ultimately, the patient may exhibit the following symptoms: (1) complete loss of motor and sensory (somatic and visceral) functions below the lesion, (2) spasticity below the lesion, (3) exaggerated deep tendon reflexes below the lesion, (4) loss of superficial abdominal and cremasteric reflexes below the lesion, (5) bilateral Babinski signs, and (6) clonus in both extremities (Adams, 1973; Snell, 1980; Carpenter and Sutin, 1983).

B. Cytological Changes

i. The perikaryal response to axotomy

The perikaryon (cell body) is the trophic center of the neuron; therefore, it is essential for the growth and maintenance of the axon.

When the axon is damaged, profound changes take place within the perikaryon. These perikaryal changes vary from one neuronal type to another and are different in any one neuron under various circumstances (Grafstein, 1983). The nature of the perikaryal response and the way in which this response relates to axonal regeneration will now be considered. Although some of the information has been gathered from studies involving neurons of the peripheral nervous system (PNS), the general principles may be applied to neurons of the CNS as well.

Typical cellular changes that take place following axotomy include swelling of the perikaryon, migration of the nucleus to the periphery, and dispersion of the basophilic Nissl substance (Lieberman, 1971; Kelly, 1981; Grafstein, 1983). Swelling of the perikaryon has been correlated with a change in membrane permeability. It has been reported that shortly after axotomy, there is a decrease in (Na^+, K^+)-activated adenosine triphosphatase (ATPase) activity in the region adjacent to the lesion (Krikorian, et. al., 1982), with depolarization of the membrane at the lesion site. This localized depolarization continues for several hours (Evans and Saunders, 1974), and evokes current flow from intact regions of the cell, resulting in a general lowering of membrane potential (Grafstein, 1975). The altered membrane potential is believed to affect the movements of some ions and water across the membrane. However, the hypothesis that water movement across the membrane is responsible for the initial swelling observed in some axotomized neurons remains debatable. Nevertheless, the phenomenon of swelling itself implies possible alteration of membrane permeability, be it the result of membrane depolarization or some other factors (Lieberman, 1971).

The dispersion of basophilic Nissl substance begins centrally in the perikaryon (central chromatolysis) and radiates outward. Chromatolysis usually lasts for 1-3 weeks (Kelly, 1981), and reaches its maximum extent 12 to 14 days following axotomy (Lieberman, 1971; Carpenter and Sutin, 1983). Different types of neuron exhibit different chromatolytic changes in response to axotomy (Lieberman, 1971; Kelly, 1981; Carpenter and Sutin, 1983; Grafstein, 1983). Chromatolysis does not occur at all in certain types of neuron, such as thalamic, locus ceruleus, and Purkinje cells, following axotomy (Grafstein, 1975, 1983; Kelly, 1981). In contrast, marked chromatolysis is observable in certain axotomized CNS neurons that apparently do not regenerate (Grafstein, 1983). It is postulated that some of the variability in the response to axotomy is a result of the various types of reactions that occur within the perikaryon subsequent to axotomy: there may be catabolic processes that deal with remodeling of the neuron, or there may be anabolic processes that deal with maintenance or recovery of the neuron (Grafstein, 1975, 1983).

A characteristic change in metabolism observed in axotomized neurons involves RNA. Cytochemical and radioisotopic studies have indicated an increase in the total quantity of RNA in the perikaryon (Murray and Grafstein, 1969; Lieberman, 1971; Murray, 1973; Whitnall and Grafstein, 1983). There may also be an occasional increase in the nucleolar size (Lieberman, 1971). Besides alterations in the amount of RNA, there are also alterations in the organization of RNA. The rough endoplasmic reticulum (RER), which constitutes the Nissl substance, dissociates during chromatolysis with shortening of the individual cisternae of RER and disorganization of the parallel arrangement of RER;

in addition, there is an increase in the proportion of free to membrane-bound polysomes (Lieberman, 1971; Grafstein, 1975; Brodal, 1981; Carpenter and Sutin, 1983). The free polysomes may further dissociate into single ribosomes (monosomes) (Lieberman, 1971; Barron, et. al., 1975). Presumably, these changes represent either an alteration in the kinds of protein synthesized on the RER, or a decrease in such synthesis (Grafstein, 1983).

An interesting observation is the increase in DNA synthesis in the perikarya of injured neurons. Watson (1965) reported that the uptake of DNA precursors was increased in some injured neurons, indicating an increase in DNA synthesis. Nuclear binding of actinomycin-D, an inhibitor of RNA synthesis that acts by intercalating into the guanine-cytosine base pair of the DNA duplex, was shown to be increased (Watson, 1974c). Furthermore, application of actinomycin-D to the perikaryon within 12 hours of axotomy has been shown to interfere with the development of the chromatolytic reaction (Torvik and Heding, 1969; Torvik and Skjorten, 1974).

Studies using radioactive protein precursors have shown that increased protein synthesis is also a characteristic response of the neuron to axonal injury (Watson, 1968; Murray and Grafstein, 1969; Ikeda and Campbell, 1971; Grafstein, 1975; Luttage, et. al., 1975; Wells and Bernstein, 1977, 1980; Grafstein, 1983). Reportedly, there is a significant increase in the synthesis of tubulin following axotomy (Giulian, et. al., 1980). Other studies have, however, produced conflicting results (Engh, et. al., 1971; Kung, 1971). It should be noted that synthesis of growth-associated polypeptides (GAPs) in mammalian CNS neurons remains quiescent after axon injury. This is significant

because studies have claimed that the failure of mammalian CNS neurons to express the GAP genes may underly the failure of CNS axons to regenerate after axonal injury (Skene and Willard, 1981). Furthermore, the increased RNA synthesis may not always lead to increased protein content because there may be an increase in proteolytic activity, as shown by a proliferation of lysosomes and an increase in acid phosphatase activity (Matthews and Raisman, 1972; Rao, et. al., 1972; Krikorian, et. al., 1982). Moreover, there is a decrease in the amount of neuronal proteins associated with neurotransmitter synthesis and degradation. For instance, in cholinergic neurons, the levels of cholinesterase (Lieberman, 1971; Krikorian, et. al., 1982) and choline acetyltransferase (Hebb and Waites, 1956) are reduced. In noradrenergic neurons, there is a decrease in the amount of dopamine-B-hydroxylase (Kopin and Silberstein, 1972; Reis and Ross, 1973; Ross, et. al., 1978), tyrosine hydroxylase (Cheah and Geffen, 1973), and monoamine oxidase (Karonen, 1964; Cheah and Geffen, 1973; Naftchi, et. al., 1980). Other studies have shown a decrease in the levels of norepinephrine (Magnusson, 1973; Ganong, 1979; Singer, et. al., 1981), dopamine (Magnusson, 1973), serotonin (Oliveras, et. al., 1977; Ganong, 1979), gamma-aminobutyric acid (GABA) (Wall, 1964; Davidhoff, 1972; Crane and Peterson, 1974; Charlton, et. al., 1981) — although Rizzoli (1968) did not find a decrease in GABA concentration — glutamate (Rizzoli, 1968), and substance P (Kanazawa, et. al., 1979) in the degenerating motor pathways of traumatized spinal cord. A further decrease in the utilization of these neurotransmitters has been thought to be a result of decreased physiological activity due to the withdrawal of presynaptic boutons from the axotomized cell (Watson, 1974a). These findings suggest that there is a change in the relative amounts of

materials synthesized by the neurons, representing a shift from the production of neurotransmitters toward the production of structural constituents presumably required for the recovery of the neuron (Krikorian, et. al., 1982). The nature, extent, and rate of these alterations depend on the type, or degree of maturation, of the neurons involved, the type of injury, and the location of the injury (Matthews and Raisman, 1972; Carpenter and Sutin, 1983; Grafstein, 1983). A lesion of the axon near the perikaryon may evoke more prominent changes, sometimes resulting in death of the perikaryon (Barron, et. al., 1976; Carpenter and Sutin, 1983) and degeneration of the proximal portion of the axon.

ii. Reactions of axons following spinal cord transection

The mammalian spinal axons react to trauma quickly and vigorously. The pathological changes that occur in the traumatized spinal axons, whether produced by transection, compression, or contusion, are similar (Pettegrew and Puchala, 1976). In order to formulate effective treatment of traumatized spinal cord, it is essential that we understand these pathological changes. Following is a synopsis of reactions of axons, which is based largely on Kao and his co-workers' observations (1977a, 1977b, 1977c). It must be emphasized that many of Kao and his co-workers' observations are controversial and, therefore, should be carefully evaluated.

It has been proposed that immediately following a transection of the spinal cord, axoplasm escapes from both the proximal and distal portions of some of the cut axons (Lubinska, 1956; Kao, 1980). The extent of axoplasmic loss varies from one fiber to another, but is generally greater in larger myelinated fibers. In contrast, the small fibers, whether myelinated or unmyelinated, show little if any loss of

axoplasm (Kao, 1980). The escaped axoplasm includes segments of neurofilaments which have probably been fragmented by a calcium-activated protease (Schlaepfer, 1982). Presumably, as a result of axoplasmic leakage, a gap is created within the axoplasmic column beginning at the cut end. One hour after the transection, the proximal and distal ends of the axons have retracted from the transection site, and both ends are separated by 1-2 mm or more from the transection site (Kao, 1980). The axoplasmic leakage stops within a few hours of the transection. Electron microscopic observations indicate that the tip of an axon is lined by axolemma within 1 hour; in addition, layers of collapsed myelin form a septum in front of the axonal tip (Kao, et. al., 1977b). It is suggested that these barriers prevent further axoplasmic leakage from the ends of the axon.

At about 3 hours after axonal transection, the axon becomes swollen and irregular in shape. It is postulated that axoplasmic material, such as mitochondria, vesicles, multivesicular bodies, neurofilaments, and neurotubules, accumulate at the cut ends of the axon (Zelena, et. al., 1968; Kao, 1980; Kelly, 1981). These swollen axonal ends are generally referred to as "terminal clubs" (Martinez and Friede, 1970). Both the proximal and distal ends swell because axoplasmic transport is bi-directional. The myelin sheath surrounding the terminal club then distends to form a spherical or oval cyst termed a "myelin microcyst" (Martinez and Friede, 1970).

Supposedly, about 1 day after axonal transection, the pressure within the microcyst reaches a level beyond which the microcyst will burst (Kao, 1980). As the microcyst bursts, the terminal club may also burst; consequently, the continuity of the axoplasmic column is disrupted

again as the axoplasm escapes from it. The escaped axoplasm disintegrates in the periaxonal environment (Webster, 1962). The subsequent terminal club, which forms at the new end of the axon, is situated farther away from the transection site than the original terminal club. This process of axonal retraction continues for approximately 1 week after the spinal cord transection and eventually ceases when the terminal club forms but does not rupture. However, it may continue for a considerable time in some axons, and fragments of degenerating axons have been found as late as 3 or 4 weeks after the trauma (Carpenter and Sutin, 1983). Consequently, in these axons the tips could be set as far as 1 cm from the site of the original transection (Kao, 1980). According to Kao and Chang (1977), terminal club rupture is significant because among the axoplasmic material released are lysosomes which contain more than 50 hydrolytic enzymes. They suggest that the activation of the released lysosomal enzymes then leads to hydrolysis of the surrounding spinal cord tissue, resulting in liquifaction necrosis of the spinal cord (Kao and Chang, 1977). This autolytic process is called "lysosomal spinal-cord autotomy" (Pettegrew and Puchala, 1976). It should be realized, however, that necrosis of the neuropil may also be caused by ischemia and hypoxia at the lesion site (see Section IV, part F).

With regard to the axon as a whole, both the proximal and the distal ends of the transected axon react similarly to the trauma. Degeneration spreads in both directions along the axon from the transection site, but only for a short distance in the proximal portion, depending on the severity of the injury (Jewett and McCarroll, 1980; Kelly, 1981; Chusid, 1982). In a clean cut only one or two internodes may be involved within the proximal stump (Carpenter and Sutin, 1983).

In the distal axon, however, Wallerian degeneration occurs, i.e., the axon and myelin sheath completely disintegrate. The myelin is broken up into elongated ellipsoid segments, which in turn fragment into smaller spherical granules. These changes generally reach their maximum 10 to 20 days after injury. The lipid globules formed are gradually converted to fatty acids, and are eventually reabsorbed (Brodal, 1981). Because blood vessels are usually disrupted by the lesion, macrophages from the systemic circulation can enter the injured area and phagocytize axonal debris (Kelly, 1981). As a rule, this disintegration occurs simultaneously throughout the length of the distal fibers, including the terminal bouton.

In summary, it appears that the failure of regeneration cannot necessarily be due to a lack of regenerative capability of the spinal neurons. We must consider the histological and biochemical characteristics of the myelin sheath and the node of Ravier which may predispose any attempt at axonal regeneration to self destruction (Kao, 1980). To facilitate spinal-cord repair, necrosis of the neuropil at the lesion site must be controlled.

iii. Axonal sprouting in the spinal cord

The exact mechanism by which the mammalian CNS reacts to injury remains somewhat of a mystery. However, numerous studies have implicated the phenomenon of axonal sprouting as a means by which recovery of function may be accomplished.

Axonal sprouting has been observed following lesions in certain parts of the adult CNS, but not in others. Liu and Chambers (1958) and Goldberger and Murray (1982) found collateral sprouting in the cat

spinal cord, and Bernstein and Bernstein (1971) reported it in the rat spinal cord. Moore (1974) determined the time course of collateral sprouting of adrenergic septal afferents, and Lynch, et. al., (1974) mapped the distribution of new synaptic terminals from collateral sprouts of intact axons in the partially denervated hippocampus. On the other hand, Kerr (1975) and Beckermann and Kerr (1976) failed to find collateral sprouting at the level of overlap between trigeminal tract fibers and those of the dorsal spinal column. It is not clear why such discrepancies exist, but it is possible that differences in experimental design or interpretation of results may be responsible. For instance, increased density of an axonal projection may be interpreted as sprouting by undamaged axons when it is actually due to shrinkage of tissue as a consequence of loss of axons and neuropil (Goldberger and Murray, 1982). Regardless of the conflicting experimental results, some researchers have considered axonal sprouting to be ubiquitous in the adult CNS, while others believe it never occurs (Goldberger and Murray, 1982).

The phenomenon of axonal sprouting is certainly of great importance when considering possible mechanisms of functional recovery subsequent to CNS lesions. Monoaminergic axons have been observed to regenerate for 5 to 10 mm through spinal cord tissue; however, the nature of their neuroglial ensheathment is not known (Bjorklund and Stenevi, 1971). At first glance, the study by Liu and Chambers (1958), in which several dorsal roots or the corticospinal tract of cats were cut ipsilaterally, appears to demonstrate that sprouts as long as 1 mm might be possible because intact axons of the remaining dorsal roots might send sprouts into the deafferented dorsal horn. Later workers (Imai and Kusama, 1969; Raisman and Field, 1973; Goldberger and Murray,

1982) have shown, however, that intact afferent fibers already exist in regions which Liu and Chambers found reinnervated by sprouts. Presumably, Liu and Chambers were observing a change in the staining properties of existing fibers rather than the arrival of newly formed sprouts (Liu and Chambers, 1980). In other studies of axonal sprouting (Raisman, 1969; Goldberger and Murray, 1982), the extent of the sprout may be as little as 1 μm, since intact axons are always present close to the observed new sprout. Furthermore, it has been demonstrated that when one source of afferents to a neuron is destroyed, adjacent pre-terminal axons of intact systems will send out collaterals to cover that part of the postsynaptic thickening previously occupied by its neighbor (Raisman, 1969; Raisman and Field, 1973; Westrum, 1974).

It is clear from the studies of collateral sprouting in the spinal cord that all of the denervated areas of the neuronal postsynaptic membrane is not occupied by collateral sprouts, even though the sprouting is denser than in normal and more extensive in its distribution (Liu and Chambers, 1980). It is also clear that sprouting from intact fibers usually occurs within the immediate vicinity of the partially denervated target areas where both the intact and the interrupted sets of axons normally overlap or terminate (Tsukahara, 1981; Goldberger and Murray, 1982). In addition, collaterals of intramedullary dorsal root axons do not sprout across the midline (Liu and Chambers, 1958) or into those regions with the strictest topographical organization, e.g., dorsal roots innervating the cell nests of nucleus gracilis will not sprout into the partially denervated nucleus cuneatus (Goldberger and Murray, 1982). Furthermore, the capacity of a particular pathway to sprout may also be regulated by a competitive or hierarchical relationship

among the various remaining intact pathways (Zimmer, 1974; Goldberger and Murray, 1982). Although no correlation has been firmly established between the extent of collateral sprouting and the extent of denervation of a given afferent input, the prominent collateral sprouting seen by Bernstein and Bernstein (1973) in the rostral end of the transected cord indicates that sprouting can occur in extensively denervated neuronal regions. However, it has also been observed that removal of ipsilateral cochlear input to one dendrite of a medial superior olivery neuron of the pons did not induce any collateral sprouting from the contralateral cochlear input (Liu and Chambers, 1974). It is postulated that the presence of abundant glial processes at the lesion site accounts for the lack of axon sprouts in the latter observation (Liu and Chambers, 1980).

Thus the phenomenon of axonal sprouting has been well researched, and it appears that axonal sprouting may be a common property of the vertebrate CNS. Studies suggest that axonal sprouting is related to functional recovery in adult mammals after CNS lesions (Goldberger and Murray, 1974, 1982; Loesche and Steward, 1977). However, it is not clear whether such regeneration leads to appropriate reinnervation of target neuronal centers denervated by the lesion. Studies by Tessler, et. al., (1980) presented some evidence for appropriate chemical specificity (substance P) in the cat dorsal horn after complete unilateral lumbosacral dorsal rhizotomy. Two additional questions may also be asked regarding collateral sprouting in the mammalian CNS:

(1) Does collateral sprouting, or a comparable process, occur in the intact normal nervous system?

(2) What mechanism induces intact axons to sprout when adjacent axons are degenerating?

Until such questions are fully explained, the phenomenon of axonal sprouting merits further research and discussion.

C. Glial Cell Response and Scar Tissue Formation in the Transected Spinal Cord

When the spinal cord is transected, axons degenerate, and collateral sprouting from intact axons may occupy denuded synaptic sites on neurons. More often, however, glial cells move in to cover them. It has been demonstrated that glial cells are important in providing trophic factors essential for axonal sprouting and regeneration (Varon and Bunge, 1975; Lindsay, 1979). However, glial cells have also been implicated in obstructing axonal regeneration in the CNS following injury. It is hypothesized that the substantial glial hyperplasia in response to spinal injury may create a physical barrier that thwarts the regenerating nerve fibers (see Section III, part C).

It has been proposed that subsequent to spinal cord transection, glial and connective tissue scar formation begins with the degeneration of most of the parenchyma of the cord at the lesion site and an influx of macrophages and hematogenous cells in regions extending 1-2 mm above and below the lesion site (Reier, et. al., 1982). While some of the macrophages may be derived from microglia, the majority are probably of hematogenous origin (Flint and Berry, 1973; Wakefield and Eidelberg, 1975; Ling, 1978). The macrophages phagocytize the degenerating debris at the lesion site, while infiltration of other mesodermal elements into the lesion site establishes a dense collagenous matrix. There is evidence that astrocytes remove debris (Lampert and Cressman, 1966; Vaughn and Peters, 1968; Cook and Wieneiwski, 1973; Nathaniel and Nathaniel,

1973; Stensaas, 1983), and are actively engaged in repairing breaches in the blood-brain and cerebrospinal-brain barriers (Berry and Riches, 1974). The other types of macroglia, i.e., the oligodendrocytes, are involved in the myelination of regenerating axons (Blakemore, 1974, 1981; Stensaas, 1977), and have been suggested to play a phagocytic role, based on ultrastructural studies (Colonnier, 1964; McMahan, 1967; Nathaniel and Nathaniel, 1973).

Subsequent to a spinal cord transection, the glial cells exhibit an increase in dry mass and RNA synthesis (Watson, 1974b). The astrocytes not only get larger, but the number and thickness of their processes increase as well. The steady accumulation of astrocytic processes and glial filaments eventually results in the formation of a distinct partition between the cut ends of the spinal cord. In addition, reactive astrocytes elaborate dense bundles of cytoplasmic processes that envelop exposed surfaces of the cord by reconstituting a glial limiting membrane (Kao, et. al., 1977a; Guth, et. al., 1981; Reier, et. al., 1981).

Reportedly, the microglial cells proliferate around chromatolytic neurons (Blinzenger and Kreutzberger, 1968; Kelly, 1981) with a significant intracellular accumulation of glial intermediate filaments that are composed of the astrocytic-specific glial fibrillary acid protein (Krikorian, et. al., 1982). Reactive glial cells may displace loosened presynaptic terminals along the proximal dendrites and cell bodies of axotomized motor neurons (Blinzenger and Kreutzberger, 1968; Kelly, 1981). The invading glial cells seem to interpose between the pre- and postsynaptic elements, thereby displacing the synapses. This displacing effect has been verified by intracellular recording of

electrical potential from perikarya of damaged motor neurons (Kelly, 1981). It was found that traumatized neurons receive reduced synaptic inputs and the evoked excitatory postsynaptic potentials (EPSP) are smaller in amplitude as though synapses on the perikaryon and proximal dendrites were removed by the invading glial cells. In contrast, Nelson and Mendell (1979) reported an increase in EPSP amplitudes following spinal cord transection.

We have seen that some of the glial cell responses seem to favor the recovery of the lesioned zone; however, the end result is the formation of a dense scar (Reier, et. al., 1982) which has been considered by many to be a major impediment to successful axonal outgrowth in the lesioned mammalian spinal cord. Nevertheless, it has been demonstrated in the transected spinal cord of goldfish that the glial scar was not a barrier to the regeneration of intrinsic neurons (see Section III, part C). Thus, other factors may contribute to abortive axonal regeneration, and further studies will be required to investigate the exact effects of glial cells on axonal regeneration.

III. FACTORS WHICH ARE THOUGHT TO BE RESPONSIBLE FOR THE LIMITED REGENERATION IN TRANSECTED SPINAL CORD

A. The Lack of a Favorable Periaxonal Environment

In the mammalian PNS, Schwann (neurolemma) cell proliferation, which occurs during development, establishes a linear array of Schwann cells along the axons (Bunge, 1983); and, the axons are enclosed individually within the Schwann cell tubes. The Schwann cells are in turn surrounded by connective tissue spaces which contain few formed elements (Guth and Windle, 1970). Ramon y Cajal (1928) considered the neurolemma cells critical in guiding and nourishing the regenerating peripheral axons. Studies have produced results supporting Ramon y Cajal's observation, i.e., neurolemma cells are capable of providing trophic support to a variety of cell types whose axons course within the PNS (Wood, 1974). In addition, it is suggested that Schwann cells provide a continuing source of trophic factor for the regenerating PNS axons (Bunge, 1980). Subsequent to axotomy, the Schwann cells will be held in their tubular configuration by a framework of basal lamina and by the forces exerted upon them from the surrounding connective tissue (Guth, 1975; Kiernan, 1979; Kao, 1980; Bunge, 1983). Regenerating axons are guided toward the distal stump by the bridge of Schwann cells that grows out from both ends of an axon (Weiss, 1941). The arrangement of Schwann cells and their basal laminae appears to provide a favorable environment for the regenerating PNS axons and their guidance to peripheral end organs (Guth, 1975; Kao, 1980; Bunge, 1983; Carpenter and Sutin, 1983).

In contrast, such an organized geometric configuration does not exist in the mammalian CNS. Here, the Schwann cells and the open

connective tissue spaces are absent, and the axons and myelin sheaths are packed very closely together. The neuroglial cells and their numerous cytoplasmic processes fill most of the available space in the CNS (Guth, 1975; Kiernan, 1979). This difference between the arrangements of neurosupportive cells and basal lamina in the PNS and CNS has been thought to account for the different regenerative capacities of the two divisions of the nervous system (Ramon y Cajal, 1928; Guth, et. al., 1970; Matinian and Andreasian, 1976; Kao, 1980). The growth of central axons into non-neural grafts transplanted into the brain (Nathaniel and Clemente, 1959; Horvat, 1966, 1969; Heinicke, 1978, 1980) supports this concept since Schwann cells of nerves originally present in the grafts may proliferate and migrate to the peripheral areas of the grafts (Kiernan, 1979), thereby providing a favorable environment for axonal outgrowth of central neurons (see Section IV, part E).

This simple explanation for abortive regeneration in the CNS does not explain several observations, however. There are no data to demonstrate convincingly that the presence of Schwann cells is absolutely necessary for the regeneration of peripheral nerves (Matinian and Andreasian, 1976); in fact, recent observations demonstrate that a variety of factors are involved in the support of cultured neurons and that different types of neurons require different sustaining factors from their environment (McCarthy and Partlow, 1976; Barde, et. al., 1978; Lindsay, 1979). Although it has been shown that Schwann sheaths contribute to the parallel distribution and faster growth of transected peripheral nerve fibers (Weiss, 1936, 1944; Satinsky, et. al., 1964; Bunge, 1983), there are observations that contradict the hypothesis that Schwann cells are essential for the growth of nerve fibers in the spinal

cord. First, some axons do regenerate despite the absence of Schwann cells. For example, neurosecretory cells of the rat hypophyseal stalk can regenerate, although only among pituicytes (which are astrocytic) and not among Schwann cells of implanted autografts of peripheral nerve (Kiernan, 1971). In addition, the cellular organization of the adult CNS is basically similar in all classes of vertebrates, yet meaningful axonal regeneration occurs only in the CNS of fishes and some amphibia and immature animals. It is possible that subtle structural substrates for the guidance of regenerating axons exist in the CNS of lower vertebrates but there is no evidence of their existence (Kiernan, 1979). Furthermore, axonal regeneration can be enhanced by treatment with certain hormones and chemicals. These agents modify the periaxonal environment, but none establishes structural organization similar to that occurring in the PNS (Kiernan, 1979). Taking the above into consideration, it is difficult to accept the view that abortive regeneration in the CNS is due to the lack of Schwann cells.

B. Intrinsic Inability of the CNS Neurons to Regenerate

i. Evidence supporting the hypothesis that CNS neurons may be capable of regenerating

As one ascends the phylogenetic scale, the regenerative capacity of the CNS decreases. The observation that axonal regeneration only occurs in lower vertebrates, such as fishes and some amphibia, demonstrates that the regenerative ability may have indeed been lost in the more complex CNS of the higher vertebrates during the course of evolution. This is supported by the fact that axonal regeneration does occur in the mammalian PNS whose organization has been proposed to have changed little in the course of phylogenetic development (Kiernan, 1979). As

another example of this hypothesis, the neurosecretory neurons of the hypophyseal stalk, a phylogenetically older area, are capable of regeneration following injury (Stutinsky, et. al., 1949; Kiernan, 1971; Dellman, 1973). It has been suggested that the vertebrate hypothalamo-neurohypophyseal system evolved from the peptidergic neurons supplying the retractor muscle of the proboscis in some nemertines, which may have been the ancestor of the vertebrates and other chordate animals (Willmer, 1974). This primitive nature of the neurosecretory cell may account for the retention of regenerative capabilities which have been lost in most of the other neurons in the mammalian CNS (Kiernan, 1979).

It is generally accepted that axonal regeneration in the transected spinal cord of immature mammals is more extensive than that found in the adult mammals (Nygren, et. al., 1971; Devor, 1974; Skene and Willard, 1981). For example, Nygren, et. al., (1971), transected the spinal cord, or performed chemical axotomy with 6-hydroxydopamine, in rats 1 to 14 days old and reported extensive axonal sprouting of monoaminergic fibers. Older rats exhibited only limited sprouting. In another study, severence of the lateral olfactory tract of 3-day-old hamsters resulted in regeneration of olfactory bulb efferents. However, such growth did not take place in 43-day-old hamsters (Devor, 1974). Thus, the regenerative capacity of neurons in the CNS of immature mammals may be explained in terms of ontogeny repeating phylogeny (Kiernan, 1971). Some investigators (Hess, 1956; McMasters, 1962), however, reported no evidence of axonal regeneration in the transected spinal cord of immature mammals.

ii. Evidence supporting the possibility of regeneration in the mammalian CNS

Although it has yet to be established that axotomized neurons of the mammalian CNS possess significant regenerative capacity, several additional experimental observations suggest that mammalian CNS neurons are capable of supporting axonal regeneration. For instance, studies on axonal transport suggest that mammalian CNS neurons possess most of the basic metabolic machinery required for axonal regeneration (Lieberman, 1971; Grafstein, 1975; Grafstein and Forman, 1980). In addition, although the perikarya of neurons that supply striated muscles are found in the brainstem and spinal cord, their axons are fully capable of regeneration following injury to peripheral nerves (Kiernan, 1979).

The phenomenon of abortive regeneration itself, meticulously described by Ramon y Cajal (1928) and subsequently verified by others, demonstrates the regenerative potential of the mature CNS neurons in response to injury. This growth capacity has been investigated in numerous studies. In one experiment using rats (Richardson, et. al., 1982), autologous sciatic nerve segments were transplanted to the thoracic region of the spinal cord. Retrograde studies with HRP indicated that some of the axons detected in the grafts came from intrinsic CNS neurons whose cell bodies lay in the nearby spinal segments and that others arose from dorsal root ganglia. This experiment demonstrates the regenerative capacity of the CNS neurons when the dense glial environment of the CNS is replaced by the more favorable environment of the PNS.

Another source of evidence comes from a different experimental model, involving the transection of midthoracic spinal cord in hibernating ground squirrels (Guth, et. al., 1981). During hibernation, metabolic

processes are depressed; consequently, collagen synthesis is depressed (Billingham and Silvers, 1960). Following spinal transection in these animals, there was minimal glial and collagen scarring at the lesion site. Also, ependymal proliferation and cyst formation (cavitation) were limited. Furthermore, there was extensive regeneration of intrinsic spinal cord and dorsal root fibers. These axons grew in abundance to the interface of the spinal cord lesion where they made an abrupt right angle turn and continued growing within the cord along the interface.

These experimental observations support Ramon y Cajal's assumption (1928) that the inability of the injured CNS neurons to regenerate is not an inherent characteristic of the CNS, but rather is the result of the lack of a favorable environment. Therefore, if it could be determined what mechanical, neurosecretory, or chemotactic factors govern the regeneration of traumatized axons and perikarya, then the neural environment might be modified so as to facilitate axonal outgrowth.

C. Scar Tissue as an Impediment to Regeneration in Transected Spinal Cord

The growth of regenerating axons in the mammalian spinal cord may be hindered as a result of histological changes occurring at the lesion site. Within several weeks after spinal cord transection, a dense fibrous connective tissue scar is formed at the lesion site (Guth, et. al., 1981). The thickness and density of the connective tissue are variable. Thick bundles of tightly packed collagen fibers are sometimes seen, but a loose, net-like arrangement is more usual (Penfield, 1927; Windle, 1956; Guth, et. al., 1981). Regenerating axons (few in number) are deflected by the dense collagenous scar, and are most frequently

observed in places where the collagen forms loose networks (Windle and Chambers, 1950; Windle, et. al., 1952; Clemente and Windle, 1954; Schonheit, 1968). The axons are occasionally seen in close association with thin strands of connective tissue (Fertig, et. al., 1971). Since the regenerating axons are usually confined within the lesion zone, it was suggested that the bundles of collagen form a meshwork that interferes with the outgrowth of axons (Windle, 1956).

In addition to collagenous scar tissue formation, there is also substantial glial cell proliferation following spinal cord transection (see Section II, part C). This glial cell proliferation, involving mostly astrocytes, is most prominent at the interface between the lesion and the adjacent intact spinal tissues, and entails the formation of dense aggregates of interlacing cytoplasmic processes (Windle, et. al., 1952; Windle, 1956; Guth, et. al., 1981; Reier, et. al., 1981; Krikorian, et. al., 1982). Since the regenerating fibers do not extend beyond the lesion site and often terminate in the matrix of glial processes, it has been postulated that the glial scar constitutes a major physical barrier to axonal regeneration (Penfield, 1927; Ramon y Cajal, 1928; Brown and McCouch, 1947; Davidhoff and Ransohoff, 1948; Windle and Chambers, 1950; Windle, 1956).

i. Observations refuting scar tissue as an inevitable inhibitor of axonal regeneration

Enhancement of axonal regeneration into the lesion zone was not obvious when the connective tissue scar was reduced by placing a synthetic dural sheath over the surface of the transected cord (Krikorian, et. al., 1981). Similarly, regenerating axons failed to enter the lesion zone when connective tissue and glial scarring was minimized in

hibernating squirrels (Guth, et. al., 1981). In addition, when the spinal cords of frogs were transected, axons regenerated through the glial scar, although the axons were deflected by dense collagenous connective tissue (Schonheit, 1968; Reier, 1979; Bohn, et. al., 1982).

The concept of a physical barrier to axonal regeneration in mammalian CNS fails to explain the regenerative capability of monoaminergic (Nygren, et. al., 1971) and neurosecretory axons (Stutinsky, et. al., 1949; Kiernan, 1971; Dellman, 1973) and the innervation of intracerebral non-neural grafts (Heinicke, 1978; Heinicke and Kiernan, 1978).

The most convincing argument against scar tissue as a physical barrier to axonal outgrowth comes from a series of experiments in which goldfish spinal cords were transected (Koppanyi, 1955; Bernstein and Bernstein, 1967, 1969). Axonal regeneration occurred vigorously in the goldfish spinal cord despite the presence of glial scar which was similar in appearance to the reactive astrocytes of the mammals and presumably just as potentially obstructive as that found in the mammalian CNS (Biganami, et. al., 1974; Kiernan, 1979). In addition, transplantation studies involving grafted astrocytic scars in amphibian optic nerve have demonstrated that compact astrocytic scars do not inhibit axonal outgrowth from neurons that are capable of regeneration (Reier, 1979; Reier, et. al., in preparation). Thus it is apparent that collagenous connective tissue interferes with the orientation of regenerating neurites, but there is little support for the hypothesis that abortive regeneration is the result of obstructive dense glial scar at the lesion site.

ii. Observations indicating scar tissue as a barrier to regeneration axons

It has been reported that axonal regeneration was enhanced in the lesioned spinal cords of mammals treated with Piromen (a polysaccharide derived from a Pseudomonas species) and adrenocorticotrophic hormone (ACTH), chemical agents that reduce the development of collagenous connective tissue and glial membrane (Windle, et. al., 1952; Clemente and Windle, 1954; Windle, 1956; Gelderd, et. al., 1980). Another supporting observation comes from experiments involving the use of a synthetic porous nylon membrane, Millipore, that prevents the growth of scar elements. By enclosing the severed ends of the cat spinal cord in a wrapping of Millipore, terminal axon sprouts were oriented longitudinally and many of them grew a few millimeters beyond the transection site (Campbell, et. al., 1957; Bassett, et. al., 1959; Thulin, 1960). The Millipore membrane eventually became calcified, however, which ended its usefulness. The concept is also consistent with the successful regeneration of central axons severed in immature animals, in which scars are less dense than in adult animals (Ssamarin, 1926; Ramon y Cajal, 1928; Sugar and Gerard, 1940; Chambers, 1955; Stelzner, et. al., 1974). However, the significance of the relative maturity of the animal at the time of transection is questionable because the regenerating axons could have arisen from either the pre-existing neurons or from nerve cells that differentiated after the transection (Sechzer, 1974). Indeed, it has been shown in neonatal cats that sparing of function is not a result of axonal regeneration, but rather, may be dependent on the survival of pathways that develop late and thereby escape the effect of spinal damage (Bregman and Goldberger, 1982). Therefore, it is not proven that axonal regeneration occurs in immature animals.

The regenerating dorsal root axons in adult mammals represents another experimental model in which the inhibitory action of astrocytes on axonal regeneration has been demonstrated. Lumbar roots in the cat were frozen several centimeters from the cord to initiate rapid Wallerian degeneration in the central processes of the ganglion (Stensaas, et. al., 1979). One month postoperatively, regenerating axons were observed within the dorsal root but cannot be identified in central nervous tissue of the transitional zone. Histological examinations indicated that most axons did not penetrate the glial limitans; instead, at least half of the myelinated axons turned back on themselves and returned along the nerve toward the ganglion. The axons that penetrated the basal lamina were found to be in close association with the processes of reactive astrocytes forming the finger-like projections. These studies imply that astrocytes of the cat spinal cord constitute an impediment to the growth of axons having regenerative capacity.

Another supporting evidence comes from experiments involving astrocytic scar grafts in mammals. Autografts of glial scars were placed between the cut ends of transected peripheral nerves (Aguayo, et. al., 1978; Reier, et. al., 1982). One to 3 months postoperatively, a few unmyelinated and occasional myelinated axons were identified at proximal and distal ends of the graft, with the majority of regenerating axons growing around rather than within the graft. These studies indicate that reactive mammalian astrocytes appear to constitute a barrier to axonal regeneration.

D. Autoimmune Inhibition of Axonal Regeneration

Mammals are allergic to their own CNS tissue, and an autoimmune disease, experimental allergic encephalomyelitis (E.A.E.), may be induced

when homogenates of CNS tissue are parenterally administered (Paterson, 1966; Feringa, et. al., 1975). The antigens mainly responsible for causing E.A.E. are the proteins of the myelin sheath (Berry and Riches, 1974). Experimental allergic encephalomyelitis is characterized by production of perivascular inflammatory responses, glial cell proliferation (scar formation), and demyelination (Feringa, et. al., 1975; Willenborg, et. al., 1977). Since damage to the spinal cord may release potentially antigenic substances into the blood, it was hypothesized that autoimmune response analogous to E.A.E. may occur (Berry and Riches, 1974; Feringa, et. al., 1975). It was further suggested that the antibodies or sensitized cells directed against these antigens only act at the lesion site (Berry and Riches, 1974). Presumably, the ends of the severed axons take in antibodies by endocytosis and transport them to their perikarya during the early stages of regeneration (Berry and Riches, 1974; Bunge, 1977; Berry, 1979; Kiernan, 1979; Grafstein, 1983). Once inside the perikarya, the antibodies might interfere with the synthesis of structural proteins, thereby inhibiting axonal regeneration and functional recovery (Berry, 1979).

In mammals the neurosecretory neurons are located in the magnocellular nuclei of the hypothalamus. Their unmyelinated axons pass through the median eminence and the infundibulum into the neurohypophysis where they end around capillary vessels. The regions of the median eminence and neurohypophysis lack blood-brain barriers (Cappell, 1929; Wislocki and King, 1936; Carpenter and Sutin, 1983); hence, neurosecretory axons would not be expected to be auto-antigenic, or capable of inducing E.A.E. when exposed to lymphoid elements. Indeed, studies have indicated that neurosecretory axons, when severed within the

neurohypophysis, are capable of vigorous regeneration (Stutinsky, et. al., 1949; Kiernan, 1971; Dellman, 1973).

It is also noteworthy that nerve regeneration does occur in lower animals and in immature animals. Billingham and Brent (1956) and Feringa, et. al., (1975) observed that those animals in which cord regeneration does occur are capable of accepting foreign cells because they lack immunologic responsiveness. Transection of the spinal cord in many lower animals is followed by regeneration with no noticeable functional deficit (Koppanyi, 1955; Bernstein and Bernstein, 1967, 1969; Wood and Cohen, 1981; Yin, et. al., 1981, 1983). During embryology and early development of animals, there is a period when they are not responsive to any antigens (Burnet, 1961; Silverstein, 1964), and injury to the spinal cords of the embryos of higher animals is sometimes followed by regeneration and recovery of normal function (Hooker and Nicholas, 1930; Hamburger, 1955; Rzehak, 1960; Stelzner, et. al., 1974).

Other evidence comes from chemotherapy studies on certain traumatized CNS systems. It was found that the regenerating tips of monoaminergic axons secrete amines that can block the immune response between the sensitized lymphocytes and myelin antigens (Field, et. al., 1971; Carnegie, et. al., 1972). In addition, administration of 5,6-dihydroxytryptamine and 6-hydroxydopamine, neurotoxic analogues of the metabolic amine precursors, enhanced the regenerative process of monoamine axons following transection of the axons in the rat (Nygren, et. al., 1974). The experiments by Feringa, et. al., (1975, 1979b) suggested that treatment of spinal rats with the immunosuppressive drug Cytoxan (cyclophosphamide) seems to enhance long descending tract regeneration, even though histological evaluation of treated animals, as

compared with control animals, failed to reveal any evidence that the treatment was effective in decreasing the scar tissue formation. One other interesting observation is reports of the enhancement of axonal regeneration in the CNS by pyrogens (Windle and Chambers, 1950; Littrell, et. al., 1953; McMasters, 1962; Gelderd, et. al., 1980) and corticosteroids (McMasters, 1962; Fertig, et. al., 1971; Gelderd, et. al., 1980), or agents that stimulate the endogenous secretion of corticosteroids (see Section IV, parts C and D). It was proposed that one of their effects is suppression of an autoimmune response to proteins released in the traumatized spinal cord.

Several findings, however, refute the hypothesis that the autoimmune response alone inhibits axonal regeneration in the CNS. Some investigators reported no evidence of anatomical restitution across the lesion in the CNS of neonatal mammals (Barnard and Carpenter, 1949, 1950; Feigin, et. al., 1951). Many investigators attribute functional recovery that is reported by proponents of the immune hypothesis to anatomical plasticity, or to continued growth in the immature animal (Hess, 1956; McMasters, 1962; Eidelberg and Stein, 1974; Sechzer, 1974; Kerr, 1975; Bregman and Goldberger, 1982). Studies indicate that, in neonatal cats subjected to spinal cord injury, the presence of corticospinal projections (which were abolished by the same lesions made in adults) was not due to regeneration, but rather, sparing of function was dependent on late development of the corticospinal tract (Prendergast and Stelzner, 1976; Cummings, et. al., 1981; Bregman and Goldberger, 1982). Further, it is known that exposure of tissue within the PNS endoneurium to lymphoid elements can result in an autoimmune disease, experimental allergic peripheral neuritis (E.A.N.); yet, traumatized

peripheral nerves are capable of regenerating (Winkler, 1965; Yonezawa, et. al., 1968; Brostoff, et. al., 1973) even in the presence of an ongoing E.A.N. (Mervart and Kiernan, 1978).

Studies directed to test the immune hypothesis by administration of immunosuppressive drugs to spinal animals also have failed to yield positive results. ACTH and corticosteroids when given in effective immunosuppressive dosage to spinal animals failed to facilitate axonal regeneration (Berry, 1979). Other studies indicate that Cytoxan may promote spinal cord regeneration by mechanisms other than suppression of the immune response (Willenborg, et. al., 1977). Cytoxan not only inhibits the rapid replication of lymphocytes, which is induced by antigenic stimuli, but also it inhibits the replication of other rapidly dividing cells, such as macrophages (Sharbaugh and Grogan, 1976), fibroblasts, and possibly glial cells (Willenborg, et. al., 1977). Macrophages may be responsible for the destruction of neural elements after spinal cord injury. Therefore, inhibition of macrophage function subsequent to the administration of Cytoxan could reduce the extent of neural destruction, thereby facilitating axonal regeneration.

In addition, studies by Willenborg, et. al., (1977) produced no evidence of cell-mediated immunity to myelin or to whole-brain tissue even after severe trauma to the spinal cord, although minor injuries to the CNS evoked the production of humoral antibodies to some cerebral lipids (Berry and Riches, 1974; Berry, 1979). Therefore, treatment with immunosuppressive drugs probably does not induce axonal regeneration in the transected spinal cord. Moreover, several axons have been observed to regenerate into intracerebral grafts of skin, muscle, tendon, thyroid gland, and salivary gland (Heinicke and Kiernan, 1978; Heinicke, 1978).

In these grafts the blood-brain barrier is absent; therefore, antibodies or sensitized cells have free access to the tips of growing axons. The growth of central axons into these grafts, all of which were exposed to serum proteins, indicate that regeneration in the CNS is not suppressed by an autoimmune mechanism.

IV. ENHANCEMENT OF AXONAL REGENERATION

A. Enzyme Treatment

The use of proteolytic (trypsin, elastase) and mucolytic (lidase, hyaluronidase) enzymes is based on the assumption that there are two components present in the scar tissue found at the transection site: (1) neuroglial elements, and (2) mesodermal connective tissue (Clemente, 1955). Since these enzymes are capable of reducing scar tissue formation (Turbes and Freeman, 1953; Freeman, et. al., 1960; Matinian and Andreasian, 1976; Pettegrew, 1976), which has been considered by many investigators to be responsible for impeding axonal regeneration in the adult CNS, some investigators believed that axonal regeneration may be induced in the transected spinal cord by treating the spinal animals with these enzymes. The rationale was to reduce the inflammatory response and gliosis in the transected spinal cord, thereby establishing a favorable environment for the regenerating nerve fibers.

Following intrathecal administration of trypsin to dogs whose spinal cords had been hemisected, the collagenous scars and glial membranes present at the lesion site were reduced. Furthermore, it was claimed that the dogs regained sensorimotor function sooner than the untreated ones (Magenis, et. al., 1952; Turbes and Freeman, 1953; Freeman, et. al., 1960). Trypsin has also been found to reduce scar formation at the transection site in adult rat spinal cords (Pettegrew, 1976). In addition to trypsin, hyaluronidase (HAase) and elastase, alone or in combination with the other enzymes, were also tested. It has been shown that one of the most important components of connective tissue is hyaluronic acid (Ragan, 1952; Freeman, 1953; Fuks, 1958;

Kasavina, 1958). It was thought that HAase would depolymerize and break down hyaluronic acid, resulting in a decrease in the viscosity of ground substance, and contribute to an increase in tissue permeability and the subsequent inhibition of collagen fiber formation (Kasavina, 1958). Presumably, HAase softens the scar tissue, thereby promoting adequate vascularization to this highly rigid and compact tissue and thus creating a favorable environment for axonal regeneration across the lesion site (Matinian and Andreasian, 1976).

Studies in the USSR reported that rats treated with trypsin, elastase, or HAase lived longer than the untreated controls, and the incidence of infection was lower (Matinian and Andreasian, 1976). Bladder control appeared to recover earlier in the treated rats. It was also claimed that nerve impulses could be transmitted across the transection site in the treated rats, and that the hind limbs of a significant number of rats responded to stimulation of the forepart of the body. In some rats, this response was reported to be well established in 6 to 8 months. Spontaneous coordinated movements were observed 10 months postoperatively. In addition, cortical evoked potential was detected 3 weeks postoperatively. Retransection of the spinal cord abolished the evoked potentials and the impulse conduction originating from stimulation of the cerebral cortex and recorded distal to the transection. Reportedly, histological examination revealed significant reduction in scar tissue formation, improved vascularization, and limited cavitation at the lesion site of the spinal rat. Neurological staining showed the presence of regenerating nerve fibers traversing the lesion site. The functional regeneration was maintained and there was no subsequent constriction of the spinal cord by dense collagenous scar tissue. The

successful experimental results have encouraged clinical treatment of human subjects with spinal cord injury in the Soviet Union. Some success was claimed in a few patients who were completely paraplegic or quadriplegic with no bladder control. Thus, enzyme therapy appeared to be promising in the treatment of spinal patients.

Researchers in the United States, however, have not been able to duplicate the results obtained by the Russian investigators (Guth, et. al., 1978; Knowles and Berry, 1978; Albuquerque, et. al., 1980; Guth, et. al., 1980). Guth, et. al., (1978) hemisected rat spinal cords at C2 and administered HAase and trypsin on alternate days subcutaneously at the lesion site for 15 days after the hemisection. They tested regeneration by looking for a return of diaphragmatic function on the ipsilateral side but could not detect any sign of this in treated or control animals. Histological findings were also negative. Knowles and Berry (1978) repeated the work of Matinian and Andreasian (1973), using trypsin, elastase, and HAase, but could not find evidence that enzyme treatment expedited regeneration or altered the nature of the scar. Perhaps the most convincing evidence against the usefulness of enzyme treatment comes from the studies by Guth, et. al., (1980). In their studies, the methodology and treatment of the spinally transected rats were identical to the Russian protocol. It was observed that 8 out of 10 animals began to walk spontaneously within 2 to 3 weeks postoperatively, even though enzyme therapy was not administered. Histological examination of the spinal cord in these rats between 1 and 4 months postoperatively showed that a small number of nerve fibers in the ventrolateral region had not been transected. A different surgical approach was then used to ensure complete transection of the spinal cord. This was done by passing a

fine probe intradurally beneath the intact spinal cord, then transecting the cord with a small fragment of razor blade and lifting the wire through the transection site. A series of rats were then treated using trypsin, elastase, lidase, HAase, and a combination of trypsin and lidase. Control rats received only the vehicle for these enzymes. All the rats with completely transected spinal cords exhibited a persistent paralysis of the hind limbs and a variety of hind limb rigidity, regardless of whether they had been treated with enzymes or vehicle. In addition, no sensory evoked potentials could be recorded from the cortex in either the treated or the control animals. None of the enzyme treatments had any effect on the histological composition of the lesion site. Histological examination revealed no difference between the treated and control rats in the amount of glial scar, connective tissue fibrosis, cavitation, and formation of cysts seen at the lesion site. However, in animals with subtotal transection of the spinal cord, the cysts were smaller and fewer, and the cut ends of the spinal cord were better approximated. Two similar studies conducted in the United States showed basically the same negative results following spinal cord transection in rats, i.e., enzyme-treated rats could not be distinguished from control, untreated rats (Feringa, et. al., 1978, 1979a; Kosel, et. al., 1979). These observations indicate that the conclusions of Matinian and Andreasian were probably based on studies in which the spinal cords of the rats were not completely transected. Although Guth, et. al., (1980) could not state with certainty that enzyme treatment had no effect on the density of the scar, they could state that significant functional neuritic regeneration did not occur in animals with either dense or loose connective tissue.

B. Nerve Growth Factor

Nerve growth factor (NGF) is a protein originally discovered by Levi-Montalcini (1954). Organs with high NGF activity are the submaxillary glands of the adult mouse (Cohen, 1960; Varon, et. al., 1967; Bocchini and Angeletti, 1969), the prostate gland of the guinea pig (Harper, et. al., 1979), and the venom glands of snakes (Cohen, 1959; Pearce, et. al., 1972). These high local concentrations do not appear, however, to play a significant role in the nervous tissue (Thoenen and Barde, 1980). NGF has also been isolated from goldfish brain (Benowitz and Greene, 1979), mouse sarcoma, and sensory or sympathetic ganglia of rats, mice, cats, cows, and man (Cohen, 1960; Varon, et. al., 1967; Bocchini and Angeletti, 1969; Matinian and Andreasian, 1976). The most commonly used NGF is the one extracted from the submaxillary gland (Matinian and Andreasian, 1976; Thoenen and Barde, 1980).

Although NGF only affects the sensory neurons and the sympathetic nervous system, interest has focused on NGF because it may belong to a wider group of neurotrophic factors that specifically control axon growth in each neurotransmitter system (Berry, 1979; Thoenen and Barde, 1980). Specifically, it has been shown to enhance the regeneration of monoaminergic fibers into implanted somatic tissue in the adult rat brain (Bjorklund and Steveni, 1972; Bjerre, et. al., 1974; Stenevi, et. al., 1974). It appears to be a trophic factor involved in cell development, maintenance, and chromatolytic changes after injury for sympathetic neurons (Hendry, 1975; West and Bunge, 1977). Presumably, NGF produces its effects by stimulating RNA production in neurons (Bernstein, et. al., 1978). According to Thoenen and Barde (1980), however, it is very unlikely that NGF molecules exert a direct regulatory effect on the

expression of genetic information at either the nuclear transcriptional or the ribosomal translational level. It was suggested that NGF acts via second messengers, the nature of which remains to be established (Thoenen and Barde, 1980).

Observations suggest that the nerves which fail to regenerate following injury may not have available to them specific growth factors such as NGF. Taking into consideration the trophic actions of NGF on sensory and sympathetic neurons, investigators have attempted to elucidate its effects on the traumatized intraspinal neurons. Realizing the difficulty involved in keeping animals alive after complete transection of the spinal cord, Scott and Liu (1963, 1964) decided to crush just the dorsal spinal column in kittens. It was claimed that NGF administration produced a marked increase in regenerative growth of axons in the dorsal spinal column; the number of regenerating axons being proportional to the dosage applied. These histological observations were supported by electrophysiological findings: action potentials were recorded 19 mm rostral to the lesion subsequent to administration of NGF. NGF has also been shown to enhance adrenergic nerve regeneration in kittens subjected to crushing injury to their spinal cord, and the magnitude of action potentials proximal to the lesion were increased (Bjorklund and Stenevi, 1972). Furthermore, NGF has been reported to significantly increase protein synthesis in sensory neurons of spinal ganglia (Scott, et. al., 1966). Similar results were also reported in experiments involving high transections of the sciatic nerve, femoral nerve, dorsal roots, and destruction of ganglion cells in different groups of neonatal rats (Scott, et. al., 1966). Other studies revealed that following transplantation of sympathetic nerve grafts into transected spinal cords,

fluorescing nerve fibers (indicative of catecholamine) could be observed to traverse the nerve graft at the lesion site and extend into the distal portion of the spinal cord (Bjorklund and Stenevi, 1972; Lindvall and Bjorklund, 1979). These findings suggest that NGF is able to increase the degree and rate of regeneration of transected adrenergic central axons.

These experiments suggest a possible therapeutic role of NGF for the treatment of spinal patients. However, negative results have also been reported and must be given due consideration. For instance, experiments with the use of NGF in spinal rats failed to demonstrate axonal regeneration (Palladini and Alfei, 1965). In addition, there has been no direct evidence that NGF is produced in vivo in tissues relevant to the physiology of the nervous system (Thoenen and Barde, 1980). It is, therefore, necessary to continue the investigation on NGF in order to determine its site of synthesis, its mode of action, and its value in the enhancement of axonal regeneration in the CNS.

C. Pyrogens

Pyrogens are bacterial polysaccharides that have many systemic effects (Windle, 1952; Stuart, 1955; Puchala and Windle, 1977). Among these are their stimulatory influence on the hypothalamohypophyseal axis, febrile response, and direct and indirect actions on leukocytes, fibroblasts, and neuroglia. Other effects include increased vascularity, reduction of edema, increased macrophage activity, and reduction of neuroglial scar formation at the CNS lesion site. Presumably, these effects result in a periaxonal environment conducive to axonal outgrowth.

The inhibitory property of pyrogens on scar tissue formation was accidentally discovered by Windle and Chambers (1950) while investigating neural center of temperature regulation in a spinal dog. The dog (which had received pyrogen treatments) would howl each time pressure was applied to its abdomen, and electrical stimulation of its bladder elicited micturition. Histological examination of the dog's spinal cord revealed that there was little cavitation or scar formation at the lesion site. Furthermore, axons were found to traverse the lesion site, and appeared to be of central origin as well as from dorsal roots. Further investigation with Piromen (derived from a Pseudomonas species) treated cats revealed no trace of a pia-glial tissue barrier (Windle and Chambers, 1950; Windle, et. al., 1952; Scott and Clemente, 1955). Similarly, Liu and Scott (1958) observed reduced astrocytosis in Piromen treated cats following crushing injury to the dorsal spinocerebellar tracts of the cats. In an experiment using more purified forms of Piromen on 18 spinal monkeys, restoration of foot movement was reported in one of them (Windle, et. al., 1956). It was concluded that Piromen prevents gliosis from occurring, thereby allowing damaged neurons the opportunity to regenerate through the lesion site. Although significant difference in the nature of scar formation was reported in the animals receiving Piromen and in the controls, axonal outgrowth in the experimental animals was limited. Other studies using Piromen or any other polysaccharides in cats (Scott and Clemente, 1952, 1955; Freeman, 1954, 1955; Littrell, 1955; Thulin, 1960; Puchala and Windle, 1977), in dogs (Windle and Chambers, 1950; Freeman, 1954; Nesmeianova, et. al., 1960), and in rats (Freeman, 1954, 1955; McMasters, 1962) have all produced results

supporting Windle, et. al., (1956) observations that pyrogens prevent scar tissue formation at the lesion site.

Histological examinations in pyrogen treated spinal animals demonstrated the presence of regenerating fibers within the scar tissue of lateral and ventral funiculi (Windle and Chambers, 1950; Scott and Clemente, 1951, 1952, 1955; Freeman, 1952; Nesmeianova, et. al., 1960; Thulin, 1960; McMasters, 1962). This regeneration was corroborated by electrophysiological studies (Scott, 1955; Scott and Clemente, 1952, 1955; Thulin, 1960). In spite of the regeneration of some nerve fibers in spinal cats and dogs treated with Piromen, restitution of voluntary hind limb movement was not observed (Windle and Chambers, 1950). However, other studies dealing with spinal animals reported some functional recovery of the hind limbs (Scott and Clemente, 1951, 1952, 1955; Littrell, et. al., 1953; Clemente and Windle, 1954; Freeman, 1954, 1955; Littrell, 1955; Nesmeianova, 1960; McMasters, 1962; Puchala and Windle, 1977). The results obtained in spinal animals treated with pyrogens were only temporary, however; long-term studies with administration of Piromen to spinal animals yielded disappointing results (Clemente and Windle, 1954; Freeman, 1955; Littrell, 1955; Windle, 1956; Campbell and Windle, 1960; Nesmeianova, 1960). Axonal regeneration in the transected spinal cord of adult cats came to a halt after 8 or more months; and in 12 to 18 months, the experimental cats became indistinguishable from the control cats (Puchala and Windle, 1977). Histological examinations revealed the formation of a dense collagenous scar tissue constricting the cord, with the resulting destruction of the neural growth that had been made and blockage of further axonal regeneration.

Furthermore, not all investigators were able to obtain positive results from the administration of pyrogens to spinal animals. Lance (1954) observed no difference in the amount of spinal scar tissue when comparing treated and control cats, and did not detect any regeneration. Arteta (1956) also failed to observe axonal regeneration in Piromen treated cats after cord transection, although the scar tissue was reported to be of a smaller amount, less dense, and better vascularized than in the control cats. O'Callaghan and Speakman (1963) could not detect any regeneration or decrease in scarring after treating rats with Piromen. Scott and Liu (1963) noticed a very limited regeneration of spinal axons in cats treated with Piromen.

It should be realized that even in the case of positive results obtained with the administration of Piromen, only a small percentage of the experimental animals showed axonal regeneration. For instance, in Freeman's experiment with rats (1955), only 15% of the animals showed regeneration, and Scott (1955) recorded conduction of impulse in approximately 40% of the cats studied. Also, in an experiment involving 376 Piromen treated neonatal rats with transected spinal cord, only 19 rats showed return of sensorimotor function and of these rats only a small number showed any evidence of regeneration (McMasters, 1962). However, in cases of successful regeneration, the possibility that the cord had been partially transected cannot be excluded (McMasters, 1962). It is difficult to assess the inconsistency of the Piromen effect. It is possible that the regeneration and glial suppressive properties of pyrogens may not always be present to the same extent in the batches used in the studies or, perhaps, the active ingredient may have

deteriorated soon after extraction. Variability in species reaction may also account for the conflicting results.

D. ACTH and Corticosteroids

Pyrogens affect the hypothalamohypothyseal axis and increase the output of suprarenal corticosteroids in addition to their inhibitory action on scar tissue formation. Experiments were, therefore, conducted to investigate the effects of ACTH, and its induced release of corticosteroids on CNS neuronal regeneration. Administration of ACTH to rat somatomotor cortex (Clemente, 1955; McMasters, 1962) and rat spinal cord (Fertig, et. al., 1971) subsequent to injury enhanced regeneration of a small number of nerve fibers in a few experimental animals. Some return of sensation in areas below the lesion was observed in the spinal rat (Fertig, et. al., 1971). The use of corticosteroids in spinal animals produced conflicting data, however. Some investigators reported positive results (Ducker and Hamit, 1969; Black and Markowitz, 1971; Lewin, et. al., 1974; Anderson, et. al., 1974; Nacimiento, et. al., 1979) while others found no improvement (Hedeman, et. al., 1974; de la Torre, et. al., 1975; Eidelberg, et. al., 1976) in animals following administration of corticosteroids for spinal cord injury. The conflicting findings may be due to variation in the type of lesion used, severity of the lesion, species of animals used, or doses and route of administration of the corticosteroids (de la Torre, 1981). However, there is no agreement among the researchers on any one specific factor. ACTH and corticosteroids have very widespread systemic effects. The effect of ACTH is similar to that of Piromen (McMasters, 1962; Puchala and Windle, 1977; Willenborg, et. al., 1977) which has been discussed in the previous section. Corticosteroids are capable of maintaining normal concentrations

of blood glucose while keeping a normal balance of electrolytes in the traumatized spinal cord of cats (Lewin, et. al., 1974).

Experiments have demonstrated that high doses of dexamethasone (a synthetic corticosteroid) can reduce the spread of morphological damage and prevent the loss of axonal conduction and reflex activity following acute spinal cord injury in cats (Nacimiento, et. al., 1979). Corticosteroid administration reportedly can reverse the sudden drop of serum cortisol level occasionally observed 8 to 24 hours after a severe spinal injury (Lewin, et. al., 1974). Corticosteroids are also useful in maintaining the structural integrity of cellular elements, thereby stabilizing the white matter of the cord in hemorrhagic lesions (Ashford, et. al., 1966; Black and Markowitz, 1971; Lewin, et. al., 1974), as well as maintaining the normal content of potassium, the major intracellular ion (Lewin, et. al., 1974). In addition, corticosteroids are known to reduce scar tissue formation and have strong anti-inflammatory properties (Foley, et. al., 1953; Clemente, 1955; McMasters, 1962; Puchala and Windle, 1977; Willenborg, et. al., 1977), and thus may be useful in reducing the amount of edema and fibrin deposits in and around the lesion site. Some findings have suggested that the major potential of corticosteroids may be in their ability to correct the sodium and potassium electrolyte imbalance resulting from tissue edema and necrosis (Lewin, et. al., 1974). Other findings indicate that the importance of corticosteroids lies in their ability to preserve the integrity of the basement membrane of capillary vessels in traumatized spinal cord tissue (Richardson and Nakamura, 1971; Ransohoff, 1972). It has been hypothesized that the protective effect of corticosteroids on cell membranes may prevent the disruption of intracellular lysosomes which release

their proteolytic enzymes upon injury, contributing to additional tissue destruction (Ashford, et. al., 1966).

In summary, administration of ACTH and corticosteroids to spinal animals appears to facilitate the regeneration of traumatized neurons. However, not all the findings are encouraging. Administration of ACTH and corticosteroids has been found to inhibit scar tissue formation at the lesion site only temporarily. A dense scar is formed at the lesion site within 3 to 18 months in spite of long-term treatment (Matinian and Andreasian, 1976; Gelderd, et. al., 1980). Furthermore, administration of certain corticosteroids, such as cortisone (Windle, et. al., 1952; Clemente and Windle, 1954; Clemente, 1955; Gunn, 1955; McMasters, 1962), did not yield satisfactory results. While capable of inhibiting the formation of collagenous tissue, cortisone had no effect on glial elements. Moreover, there is only one instance of observed functional recovery in spinal animals administered with ACTH (McMasters, 1962). It was reported that of the 98 rats receiving ACTH, only 5 showed temporary recovery of sensorimotor function of the hind limbs. Thus, it is apparent that hard data are needed to substantiate the use of ACTH and corticosteroids in treating spinal cord injury. In particular, we need to further evaluate the effect of ACTH and corticosteroids on scar tissue formation subsequent to spinal cord injury.

E. Peripheral Nervous Tissue Transplantation Into the Spinal Cord

In mature mammals the periaxonal environment in the PNS is more favorable for regenerative axonal growth than that in the CNS. It has been well established that injured peripheral axons regenerate readily through Schwann cell tubes in the peripheral nerves (Guth, 1975; Kao,

1980; Bunge, 1983; Carpenter and Sutin, 1983). Therefore, transplantation of peripheral nervous tissue into the gap of a transected spinal cord is an attempt to restore structural and functional continuity of the cord by providing "guidance channels" for the regenerating spinal axons (Kao, et. al., 1977a; Thulin and Bunge, 1972; Zalewski, et. al., 1978; Richardson, et. al., 1982; Das, 1983).

When autologous peripheral nerve segments were grafted into the thoracic spinal cord of rats or other animals, the Schwann cell sheaths survived and were reinnervated by spinal axons (Kao, et. al., 1977a; Richardson, et. al., 1980, 1982). These peripheral nerve grafts fused with the cut ends of the spinal cord. Although there was little collagenous scarring at the graft interface, intense gliosis was observed. Subsequently, many astrocytic processes were found along the spinal cord-graft interface. Some of these formed a glial limiting membrane at the spinal cord-graft interfaces. Nodes of Ranvier, peripheral myelin, and Schwann cell cytoplasm were found on the peripheral nerve side, while oligodendrocytic myelin and astrocytic processes were found on the spinal cord side (Gilson and Stensaas, 1974; Kao, 1974; Kao, et. al., 1977a; Richardson, et. al., 1980, 1982). It was reported that the majority of the fibers detected within the graft were derived from intersegmental neurons close to the graft and from the dorsal root ganglia located at the level of the graft or as far as 7 segments caudal to the graft (Richardson, et. al., 1982). Other studies have reported similar results, i.e., axons in the peripheral nerve grafts arise from cell bodies in the vicinity of the adjacent CNS tissue (David and Aguayo, 1981; Wendt, et. al., 1981; Benfey and Aguayo, 1982). Although the nerve fibers entered the graft and extended through the graft to the

spinal cord-graft junction, none were observed to re-enter the spinal cord for any significant distance (David and Aguayo, 1981; Richardson, et. al., 1982). Presumably, the failure of axonal elongation into the cord may be due to inadequate peripheral or central neuroglial ensheathment of the axonal tip. It may also be explained by the following hypotheses: (1) Schwann cells in the PNS graft provide unique trophic support, which is not available in the CNS tissue, and (2) astrocytes inhibit axonal regeneration (see Section III, parts A and C). Since few regenerating nerve fibers re-entered the spinal cord, it is not surprising that none of the experimental animals showed any functional recovery.

It has been hypothesized that cyst-like cavities that form 1 week postoperatively at the cut ends of the cord represent a block to axonal regeneration. It has been claimed that when the damaged portion of the cord and the cysts were surgically removed without inducing further neural trauma prior to the transplantation, the cysts did not form and spinal cord nerve fibers grew into the graft (Kao, 1980). Reportedly, about 10% of the experimental dogs regained the ability to walk. Some investigators have suggested that the ability of the treated dogs to walk may be explained by a spinal reflex, originating in the cord below the lesion. As an evidence against this possibility, Kao (1980) claimed that the walking stopped when he reinjured the spinal cord of the treated dogs, rostral to the original site. Nevertheless, this report remains dubious, because it was not shown if the regenerating nerve fibers actually re-entered the spinal cord, or that neural connections were reestablished. Moreover, the claim that cavitation did not take place following the second operation is very surprising.

Thus, the therapeutic use of peripheral nerve grafts in the traumatized spinal cord to enhance functional recovery remains to be firmly established. In order to do so, we need to find out why elongating axons fail to re-enter the spinal cord. In addition, we need to further evaluate the distribution and properties of spinal nerve fibers that are capable of regenerating into peripheral nerve grafts and determine the influence of nerve grafts on the perikaryal response of axotomized neurons.

F. Hyperbaric Oxygen Therapy

Following traumatic damage to the spinal cord, blood perfusion of the cord tissue is severely impaired, resulting in cellular hypoxia and eventual cell death (Ducker and Perot, 1971; Kobrine, et. al., 1975; Griffith, 1976; Cawthon, et. al., 1980). The diminished blood flow may be due to vasospasm, or microvascular occlusions, or both (Naftchi, et. al., 1980). Cell death involves breakdown of the cell and release of lysosomal enzymes, which in turn cause dissolution of the neuropil and formation of cavities in the spinal cord at the lesion site (Gelderd, 1980). The mechanism of the usefulness of hyperbaric oxygen therapy is not well understood, but it is believed that an increase in the tissue oxygenation under pressure may alleviate ischemia, thereby reducing the degree of necrosis and the subsequent cavitation of the neuropil until revascularization of the spinal cord occurs (Hartzug, et. al., 1969; Kelly, et. al., 1974; Yeo, et. al., 1976, 1977; Gelderd, et. al., 1980).

Attempts to minimize tissue damage after spinal cord injury by the use of hyperbaric oxygenation have produced conflicting results. Improvement of motor recovery was observed in cord-injured dogs (Kelly,

et. al., 1974; Hansebout, et. al., 1981) and in spinal rats (Gelderd, et. al., 1980; Hansebout, et. al., 1981; Gelderd, 1983) subjected to hyperbaric oxygen therapy. Histological examination of the traumatized spinal cord in dogs failed to reveal any significant difference between the treated and the control dogs. The gray and white matter were severely damaged in all groups with spinal cord injury; however, treated dogs with better functional results tended to have less destruction of the white matter (Hansebout, et. al., 1981). Examination of the lesion area in hyperbaric oxygen treated rats revealed reduced spinal cord cavitation and an increase in the collagen density (Juva, et. al., 1966; Hunt, et. al., 1977; Gelderd, 1983), vascularity, and extent of the scar tissue (Gelderd, 1983). Presumably, the reduced oxygen levels subsequent to spinal cord injury prevent collagen synthesis with a concomitant intracellular accumulation of large quantities of collagen precursors. When oxygen is made available to the fibroblasts, rapid synthesis of collagen then follows (Gelderd, 1983). In spite of the increase in scar formation, the number of nerve fibers growing into the scar has been found to increase. They are arranged in tightly packed fascicles. Since the formation of scar tissue has been suggested as a physical barrier to regenerating nerve fibers, the administration of hyperbaric oxygen to spinal animals as a therapeutic measure to facilitate axonal regeneration should be evaluated carefully.

A major criticism of hyperbaric oxygen therapy lies in its ability to cause oxygen intoxication at pressures of 4 atm or above, leading to lung damage, convulsions, and eventual death of the animal (Fuson, et. al., 1965; Ogilvie and Balentine, 1973, 1975). Since such oxygen toxicity is directly related to the level of pressure and the

duration of treatment, guidelines must be established and followed during hyperbaric oxygen therapy to eliminate any possible negative effects. A method of localized treatment aimed at improving oxygenation of the injured spinal cord may be safer than a systemic hyperbaric oxygen treatment (Hansebout, et. al., 1981). In view of the positive results, hyperbaric oxygen therapy appears to be an effective method capable of reducing cavitation and necrosis in the traumatized spinal cord. Therefore, further evaluation of various forms of hyperbaric oxygen therapy should be conducted, and the effectiveness of combining hyperbaric oxygen treatment with other forms of therapy should also be investigated.

V. CONCLUSIONS

The subject of axonal regeneration and functional restitution in the traumatized mammalian spinal cord has been actively investigated. The importance of CNS regeneration research in the alleviation of human suffering is obvious. As a result of intense research in the past 20 years, we now have a better understanding of the reactions of the spinal cord to injury and of its capacity to regenerate. It is now possible to dismiss the pessimistic view that axonal regeneration in the CNS cannot be accomplished.

The view that scar tissue acts as an impenetrable mechanical barrier to the regenerating nerve fibers is no longer tenable. The process of abortive regeneration in the CNS cannot be explained on the basis of a lack of favorable environment, nor can it be declared that central axons are intrinsically incapable of regenerating. Neither is the theory of autoimmune inhibition of axonal regeneration in the CNS acceptable. Even though various chemical and physical methods used to enhance CNS regeneration have not been successful, the enigma of regeneration in the mammalian CNS should not be considered unsolvable.

The objective of future research is rather clear. Some investigators are concentrating on the perineuronal or extrinsic factors involved, such as glial cells or chemical agents that may affect axonal regeneration, while others are focusing on the intrinsic neural activities which may dictate axon regrowth. All agree, however, that both types of factors are of equal importance and deserve active investigation. Presently, several aspects of CNS regeneration are of immediate research interest. The histological and physiological attributes of

collateral sprouting must be identified. In addition, neuronal specificities and reestablishment of synaptic connections after spinal cord injury must be further investigated. The processes of protein synthesis, transport, and degradation in traumatized neurons and the regulatory mechanisms that determine the rates of these processes should also be elucidated. More important, the metabolic, trophic, or inhibitory interactions among CNS neurons, neuroglia, and vascular elements must be thoroughly analyzed. The emphasis of current studies, however, is on identifying the factors responsible for inhibiting the regenerative process of CNS neurons and on devising methods to counteract these inhibitory factors, thereby facilitating axonal regeneration in the CNS.

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