MOLECULAR AND CELLULAR ASPECTS OF NERVE REGENERATION

Author: **Michal Schwartz**
Department of Neurobiology
Weizmann Institute of Science
Rehovot, Israel

Referee: Bernice Grafstein
Department of Physiology
Cornell University Medical College
New York, New York

I. INTRODUCTION

The nervous system is a sophisticated communications network that senses, integrates, and transduces information between the external and internal milieu. The integrity of the network is essential for its successful functioning. This integrity may be disrupted by an injury, with a consequent process of degeneration that leads to behavioral abnormalities or even an irreversible loss of functional activity. The functional activity may be rescued by early prevention of degeneration or by a process of regeneration.

Various treatments have been tried to prevent degeneration.[1,2] These include cooling in an attempt to avoid ischemia,[3,4] and application of naloxone,[5,6] phenytoin,[7] puromycin,[8] corticotrophin, or triiodothyronine[9] in an attempt to avoid some of the consequences of trauma. Lately, low-energy laser irradiation (He-Ne, 630 nm) applied to both injured peripheral and central neurons has prolonged the survival of the injured fibers manifested physiologically and morphologically.[10,11]

In most injuries, however, the post-traumatic degeneration cannot be avoided and therefore recovery of the injured neuron, by reconstruction of the severed part, depends on its ability to regenerate. Neuronal regeneration means regrowth of injured axons, followed by restoration of their original synaptic connections and, finally, recovery of the original physiological functions. The neurons of different species have different abilities to regenerate. Neurons of lower vertebrates ordinarily regenerate. This also holds for neurons of the peripheral nervous system (PNS) in mammals. In contrast, most of the neurons of the central nervous system (CNS) in mammals seldom regenerate.[12-17]

The process of axonal regeneration has been suggested to involve (1) establishment of a neuronal growth state and formation of new sprouts, (2) elongation of the new sprouts, and (3) target recognition, synaptogenesis, and cessation of axonal elongation.[18] Successful regeneration probably depends on a synchronized progression of the events that are involved in these major phases. Malfunction of any of these events or lack of synchrony may hamper the process of regeneration.

Some of the events involved in nerve regeneration are reminiscent of development.[17] However, this notion should be considered with care because regenerating neurons give rise to growth in an adult environment which may require the involvement of distinct sets of neuronal substances other than those required during growth in development.

The cascade of events that leads to growth and elongation may be triggered by one signal, or each of the sequential events may be triggered by a distinct signal. Signals may be provided either by the axonal injury itself or by injury-stimulated changes in the environment. Induction of regeneration by the injury itself implies that regenerative and nonregenerative nerves differ in an intrinsic property essential for regeneration. Induction of regeneration by environmental factors implies that both the regenerative and nonregenerative nerves may be

similar, but the environment of the nonregenerative nerves lacks constituents essential for regeneration (or it contains inhibitory elements). Studies on regeneration have, therefore, been focused on identifying the factors (neuronal or environmental) that allow the neurons to shift from a mature resting state into a growth state, thereby accomplishing the subsequent events needed for regeneration.

The emphasis in this review is on nerve regeneration rather than prevention of degeneration. The cited works constitute a selective list which reflects the author's own prejudices regarding emphasis.

II. REGENERATION-ASSOCIATED EVENTS IN THE NEURON

A. Changes Related to the Cell Body

The neuronal cell body synthesizes and produces the materials required for growth of the nerve during development and injury-induced regeneration as well as for maintenance upon maturation. Injury causes metabolic alterations that are reflected in morphological and biochemical changes in the cell body. Some of the changes have been correlated with regeneration, and they do not occur in nonregenerative systems. Most of the changes are presumably needed to equip the neurons with substances necessary for axonal reconstruction, for recognition between axonal fibers and supportive cells, and for target reconnection. However, there are indications that an early response in the axon, manifested by growth initiation and adenylate cyclase activity, may occur independently of the cell body response;[19-22] such an early growth may exhaust the cytoplasmic pool of proteins.

1. Morphological Changes

After axotomy, most neuronal cell bodies that have been studied become enlarged.[23,24] There is an increase in the amount of nucleolar material, and the cells acquire multiple nucleoli. In the newt, for example, these alterations occur within 4 to 7 days postaxotomy in 50 to 60% of the retinal ganglion cells.[25] In addition, the cell bodies of some regenerative systems lose the ability to be stained by basic dyes, and their nuclei migrate to the periphery within 24 hr after injury. These microscopical changes are known as features of the chromatolytic response.[26,27] Studies in the neurons of the frog ventral horn suggested a direct relationship between the injury-induced chromatolytic response and regenerative ability. During the first 2 weeks after axotomy of the frog ventral horn, the neuronal nucleus becomes more translucent and the nucleolus becomes enlarged and less compact. The cisternae of the granular endoplasmic reticulum vesiculate and ribosomes dissociate from the membrane. Free ribosomes and polysomes are dispersed in the cytoplasmic matrix. However, such a correlation between chromatolytic response and regeneration has not been observed in other studies. For example, in the fish, injury causes an increase in Nissl staining. An increase in nuclear size begins 3 to 5 days after injury. Cell size and polyribosome content are increased as early as 6 to 7 days after injury. Hypertrophy of the rough endoplasmic reticulum (RER) occurred by day 14. Between 14 and 30 days, perikaryal volume and amino acid incorporation reach their maximum levels.[28-33] In the fish visual system, these changes are accompanied by functional recovery without a chromatolytic response. Although there is no correlation between the chromatolytic response and regeneration, distinctive responses were found to be induced in the same cell body when its peripheral branch, which is regenerable, is injured compared with injury to its nonregenerable central branch.[34] In nonregenerative systems, such as in the rat optic nerve, injury causes a transient shrinkage of the retinal ganglion cells during the first several weeks postoperation. Loss of 60% of the neurons in the ganglion cell layer occurs between 3 to 7 months after injury.[35]

2. Biochemical Changes

The morphological changes described above coincide with changes in RNA synthesis and precede changes in protein synthesis.

a. RNA Synthesis

Increased RNA synthesis in regenerative systems after axonal injury was demonstrated by incorporation of ^{3}H-uridine into cells, as evaluated by autoradiography of histological sections of the corresponding retinas.[36,37] At 4 days postaxotomy, there is an increased incorporation of ^{3}H-labeled precursors of RNA into retinal cells and an increase in labeling of total retinal RNA. This elevation begins at 2 to 3 days after injury and peaks at 4 to 7 days. In addition to changes in RNA precursor metabolism, optic nerve regeneration is characterized by alterations in labeling of free and membrane-bound retinal ribosomes as well as in post-transcriptional processing of mRNA.[38] Elevated synthesis of RNA[38,39] and an increase in enzymes involved in the metabolism of RNA were also measured in the fish optic nerve. Thus, injury of the fish optic nerve was shown to cause increased accumulation of radioactivity from ^{3}H-uridine in the corresponding retinas,[39] reflecting an increase in retinal uridine kinase and uridine mono- and diphosphate kinase, with a peak at day 4 after injury.[40] The increased RNA synthesis in the fish is correlated with the observed morphological changes manifested by the nucleolar enlargement (Section II.A.1).

b. Metabolic and Enzymatic Activities

In a regenerative system (e.g., facial and hypoglossal nerves of rat), the injury-induced increase in metabolic activity is manifested by an early increase in glucose utilization[41,42] and enhancement of polyamine synthesis.[43,44] In cases where glucose utilization is prevented, e.g., in transected nerve of hypoglycemic rat,[37] the regeneration process is retarded. Unilateral destruction of the entorhinal cortex in rat resulted in a decrease in glucose utilization in the denervated dentate gyrus between 1 to 4 days postlesion. This decrease was not restricted to the denervated portions. A dramatic increase in 2-deoxyglucose uptake was noticed 6 to 10 days postlesion and was suggested to be associated with afferent or postsynaptic elements during lesion-induced synaptogenesis.[45]

c. Protein Synthesis and Axonal Transport

Incorporation of radioactive amino acids into retinal ganglion cells is increased commencing 4 days after optic nerve injury.[30] Quantitative electron-microscopic autoradiography studies in the goldfish retina at various stages after injury revealed the increased labeling of nucleus and nucleolus at 6 days after injury, consistent with the increased synthesis of nucleoproteins. From 6 to 30 days, the proportion of total protein synthesized in the RER increased, whereas the proportion synthesized in free ribosomes decreased. The amount of newly synthesized protein delivered to the Golgi apparatus increased. Smooth membrane elements were also increased. The authors attributed the possible contribution of these elements to the increase in fast axonal transport.[46]

Neuronal substances required for axonal growth, maintenance, and function are synthesized in the cell body and transferred to the axon via axonal transport mechanisms. In the perikarya of regenerating retinal ganglion cells, the ultracellular transport of newly synthesized proteins through organelles varies with the stage of axonal regeneration.[33] The axonal transport of the various synthesized substances showed changes in type, amount, and rate. Among these are proteins, glycoproteins, RNA, lipids, and polyamine-related compounds.[47-68] In the toad optic nerve, the total radioactive label associated with the most rapidly transported proteins increased three- to fourfold during the first 8 days after injury, and there was a tenfold increase in the rate of slow-moving proteins.[67-69] In the sciatic nerve, the insertion of axonally transported glycoproteins into the axolemma of regenerating nerve

sprouts showed increased labeling 7 to 14 days after injury.[70] There is a 20-fold increase in the transport of 4S RNA in goldfish optic nerve relative to a noninjured nerve.[65,66] The transported RNA includes primarily tRNA molecules, presumably participating in post-translational modifications required for growth during regeneration[62,66] and for axonal maintenance. The polyamine-related compounds (i.e., putrescine, spermine, and spermidine) undergo increased axonal transport in regenerating optic nerves (examined in fish). It was speculated that this increase may be related to their putative trophic activities.[62,66]

Among the axonally transported polypeptides which undergo changes after injury are proteins which are conveyed by fast and slow transport. These include membrane and cytoskeletal components of abundant and nonabundant proteins.[61,67,68,71]

Among the abundant proteins, tubulin has been intensively studied.[63,72] During injury-induced regeneration,[72] there is an increase in tubulin synthesis, preferentially of the β-tubulin subunit. This suggests that β-tubulin has a role in neuritic extension. This is in line with the finding that neuritic extension from neuroblastoma cells is associated with increased synthesis of β-tubulin.[73] Along with the increased tubulin synthesis, there is a selective increase in the synthesis of two low molecular weight microtubule-associated proteins (TAU factors),[72] which are presumably associated with neurite extension. They perhaps participate in determining the spatial organization of the growing microtubules or in the stabilization of microtubules within the axon. This assumption is substantiated by the observation that both tubulin and TAU factors, unlike the high molecular weight microtubule-associated proteins (MAPs), undergo axonal transport with a similar rate.[74] The synthesis of other cytoskeleton-related proteins may also increase. These include actin, neurofilament-like proteins, and membrane-associated cytoskeletal materials.[59,60,64]

Analysis of axonally transported proteins revealed that among the rapidly transported proteins a few showed an increase of up to 20-fold during regeneration. A few of these polypeptides are hardly detectable in the mature intact neurons but appear in the developing nerves. During regeneration, they are expressed to a higher level in lower vertebrates (specifically in the visual system) and in mammalian PNS.[59] These polypeptides were therefore named collectively as growth-associated proteins (GAPs).[67,68] Among the proteins that showed an increased level during regeneration, the following were identified: a 23-kdalton protein;[67,68] an acidic 43- to 49-kdalton protein,[53] as well as 50- and 210-kdalton glycoproteins in toad and goldfish optic nerves;[51,67,68] 120- and 160-kdalton proteins in goldfish optic nerves,[53,75] and 18- and 66-kdalton polypeptides in rat sciatic nerves.[76,77] It should be noted, however, that the level of some proteins decreases during regeneration (e.g., a 13-kdalton polypeptide in rat sciatic nerves).[76,77]

No specific role has been assigned, so far, to most of the GAPs. However, the timing of appearance of distinct proteins implies that they play distinct roles in the process of regeneration. In the fish optic nerve, the levels of certain axonally transported proteins depend on the presence of the target, the optic tectum, whereas the level of others are target independent. Thus, for example, when the optic nerve fibers of the injured axons are allowed to contact the tectum, the amount of 120- and 160-kdalton rapidly transported proteins is increased, whereas the 26-kdalton protein reverted to normal values and the group of the acidic 43- to 49-kdalton proteins remained unaffected by the target.[53,75] Prevention of contact with the tectum caused the 26-kdalton protein to remain high. These results imply that the appearance of some of the transported proteins, whose level is associated with growth, might be the result of synaptogenesis and therefore related to the cessation of growth. This notion is further substantiated by the observation that in the absence of the primary target the regenerative response (manifested morphologically and biochemically) is prolonged.[75,78-80] Thus, while 3 weeks after crush when accompanied by reconnection with the contralateral target, cell size and the axonal transport rates begin to return to normal, the cell body reaction is still manifested 10 to 12 weeks after injury in the absence of the target.[80]

Changes in protein synthesis (putative GAPs) may appear in nonregenerative systems in response to artificially imposed external signals.[81,82] They ordinarily do not appear in such systems after injury.

The changes in protein synthesis in regeneration were found to be reflected also by changes in translation products of mRNA, derived from the tissues in which the cell bodies are located. This has been demonstrated so far in goldfish retinas with injured optic nerves[72,83,84] and in rabbit retinas with injured optic nerves which were exposed to environmental modifications.[84] In the case of tubulin, the amounts of both the protein and the mRNA (using a cDNA probe specific for tubulin) encoding for the protein could be directly measured. The increase in protein was reflected by an increase in RNA.[72] In no other cases could the amount of the mRNA, encoding for a specific protein, be directly measured.

d. Kinetic Parameters of Axonal Transport and Growth

Changes in protein synthesis occurring after axotomy are accompanied, in some cases, by changes in the rate of transport of the proteins. In the rat sciatic nerve, after a delay of 32 days, axonal growth advances at the rate of 3.0 ± 0.1 mm/day.[85] In mouse sciatic nerve, for example, the rate is increased by 27% following a conditioning lesion.[86] In the goldfish optic nerve, following a testing lesion, histological studies revealed an increased rate of growth (0.74 ± 0.13 vs. 0.34 ± 0.03 mm/day).[87]

Application of the calcium ionophone A-23187 to the site of injury of fish optic nerve or increased Ca^{2+} entry significantly enhanced the rate of recovery.[88] Thus, in the goldfish optic nerve, the transport rates and the amounts of both the fast and slow axonally transported components increase during regeneration.[89-92] In most cases, however, the rate of anterograde transport remains unchanged during regeneration, and the major quantitative changes are manifested by the amount of the transported material. The issue of changes in anterograde transport during regeneration should be interpreted with caution since comparison among different studies is difficult. The difficulties evolve from the fact that variation in species, nerve type, type of trauma, distance from lesion to cell body, and labeling techniques may give rise to different results.[92] An example of the possible association between injury-induced changes in kinetics and regeneration was shown when adult rat dorsal root was injured as compared to injury of the peripheral branch. While in the former the rate of axonal flow was retarded, it was accelerated when the peripheral branch was injured.[94]

Regeneration is also accompanied by changes in retrograde transport. In the rat sciatic nerve, the modified transport rate persists for the first 5 days following the injury. The transport rate returns to the original state at the end of this period, whereas in nonregenerating nerves the retrograde transport remains altered.[93] In the noninjured goldfish optic nerve, 40% of the glycoprotein reaching the optic tectum is retrogradely transported to the cell body.[95] The retrogradely transported material may differ in composition[77] and velocity[96] from those which arrived anterogradely. The amount of retrogradely transported glycoproteins is greatly elevated 2 weeks after cut of the optic nerve tract.[95] It is suggested that changes in retrograde transport during regeneration may be needed for eliciting the appropriate response in the cell body and for informing the cell body of the progress of regeneration.[77,95-99] In the fish optic nerve, the nerve growth factor (NGF) is retrogradely transported into the retinal ganglion cells only when applied to injured optic nerves since no NGF receptors are available.[100]

e. Lipids

The amount of labeling and the transport of lipids, phospholipids, and gangliosides are increased in the fish optic nerve after injury[101-104] and in regenerating peripheral nerves of mammals.[105] Gangliosides were shown to be involved in growth and regeneration both in vivo and in vitro.[106-108] When [^{3}H]mevalonolactone was used for *in situ* labeling of choles-

terol, about a fourfold increase in the amount of labeled cholesterol transport was measured during regeneration of fish optic nerve, with no changes in labeling in the retina.[109] In vitro studies using the neurite outgrowth from regenerating retinas as a model[109] pointed to the possible requirement for cholesterol biosynthesis for neurite outgrowth. Addition of lipids such as phosphatidylcholine, phosphatidylserine, and cholesterol, presumably fluidity-modulating agents, modified the neurite outgrowth from these cultures.[110] Indications for an increase in lipid metabolism in regeneration, also changes in lipid synthesis and degeneration, emerged from recent studies which identified a polypeptide (37 kdaltons) derived from nonneuronal cells of rat sciatic nerve involved in lipid metabolism as a form of apolipoprotein E[111,112] and a 28-kdalton polypeptide in regenerating fish optic nerve as apoliproprotein A-I (Harel, Fainaru, Safer, and Schwartz, submitted). The presumption is that this protein is involved in either lipid degradation or biosynthesis.

B. Changes Associated with the Axon

Within 24 hr after injury, the axons of regenerating neurons develop terminal bulbs containing tangles of neurofilaments surrounded by an accumulation of various cellular organelles. Subsequently, new sprouts emerge and elongate.[113,114] The sprouts are basically extensions of cytoplasm from the cell body bearing specific structures at their tips called growth cones. The tips have a special cytoskeletal ultrastructure which distinguishes them from other parts of the nerve fiber.[115] The tips of neuritic sprouts (i.e., growth cones) are believed to be the major sites of membrane assembly.[116,117] The tips are motile and thus they constantly explore and interact with the environment, adhere to the substrate on which they are growing, sense tropic and trophic influences, and interact with other cells.[118-122]

Neurite sprouting can be initiated in regenerative systems by axotomy, degeneration of nearby nerves, injection of a neuron-specific toxin, and target degeneration. Sprouting and elongation may be accelerated in neurons that had a prior axonal injury, i.e., a conditioning lesion.[123,124] In some nonregenerative systems (e.g., the rat optic nerve or spinal cord) injury results in abortive sprouting.[125,126]

The extensive growth of new fibers after axonal injury is also reflected in vitro. Neurons of regenerative systems (e.g., goldfish optic nerves) can send out neurites in culture in response to injury.[127-131] In contrast, injury of axons of the mammalian spinal cord or brain does not lead to sprouting in culture.

III. THE RECIPROCAL RELATIONSHIP BETWEEN THE NEURON AND ITS MICROENVIRONMENT

The biochemical changes that occur in a regenerating neuron are associated with growth and elongation. It is not yet known which of these changes are prerequisite, facilitating, or causal for regeneration. The lack of these changes, including the obligatory ones, in nonregenerative nerves may stem from a malfunction (or lack of function) of the system in which prior events eventually lead to these changes. Such events might occur in the neuron or its microenvironment.

A. Environmental Effects on the Neuron

In intact nerves, there is a mutual relationship between the axon and the nonneuronal cells. This relationship is presumably required for maintenance and functional activity of the axon and the fully differentiated nonneuronal cells. As a result of an injury, this mutual relationship is disrupted with a consequent transition of the environment to one that is either regeneration supportive or inhibitory.

Axotomy induces changes in the state of growth of the surrounding nonneuronal cells.[132-138] For example, morphological studies of astrocytes after injury provide clear evi-

dence that astrocytes are functioning as macrophages. Selectively, the subpial astrocytes accumulate glycogen and an abundance of microfilaments.[135] These changes may lead to formation of a hostile environment due to the appearance of either scar tissue (made of collagen or glia) or axonal growth inhibitors.[139] Thus, attempts were made to attribute the regenerative failure of axons in the adult mammalian CNS to the release of growth inhibitors from injured oligodendrocytes and/or myelin. Accordingly, the growth capacity of non-myelinated injured CNS fibers serves as an indication for this hypothesis since no oligodendrocytic breakdown products of myelin are expected.[139] Alternatively, the proliferation of the glial cells in a regenerative system may lead to formation of a growth-supportive environment.[81,140-142] Surgical manipulations aimed at determining whether a dense glial scar, which is formed by the proliferating glial cells, interferes with outgrowth of neurites in regeneration showed that such a scar does not represent a major obstacle.[143,144] However, there is no conclusive information regarding the contribution of scarring to the failure of regeneration in the mammalian CNS.[145-147] It is possible that the lack of regeneration may not stem from hostile or ineffective glial cells, but from a deficiency in reactive glial cells[148] or the inability of the reactive glia to provide the appropriate element needed for growth. Nevertheless, these cells may be developed into supportive cells by an appropriate intervention at the right time. It appears that the formation of both the hostile and the supportive environments may occur, each at a different time.[136] The net outcome of these opposing contributions may have an impact on the direction of the response to the injury, i.e., regeneration or degeneration.

Nerve-transplantation experiments demonstrated that the neuronal environment should be supportive in order to allow regeneration to occur.[149-152] Injured spinal and brain axons regenerate readily through grafted columns of Schwann cells in peripheral nerves but seldom enter grafted CNS nerve segments.[149-155] This indicates that, in contrast to glial cells, the nonneuronal cells of the peripheral nerves (e.g., the Schwann cells) or components associated with them (e.g., diffusible or extracellular matrix) have the appropriate properties needed for regeneration.

1. Nonneuronal Cells

The PNS environment consists primarily of fibroblasts and Schwann cells, whereas in the CNS the environment consists of oligodendrocytes, astrocytes, and microglia. Although the cellular constituents of the CNS environment in lower vertebrates and mammals are similar, the behavioral similarities in these classes of organisms are between the mammalian PNS and the CNS of lower vertebrates.[156-158] For example, glial channeling in lower vertebrates resembles, in some aspects, channels formed by Schwann cells in the mammalian PNS.[159-161] This similarity is further supported by other studies which demonstrate a possible role for glial cells in guiding regenerating axons,[162] as is the role of Schwann cells in regenerative peripheral nerves.[163] Thus, for example, rat sciatic nerves exclusively regenerated toward nerve grafts containing the distal segment Schwann cells. The effect was attributed to the cells and to diffusible molecules active at a distance of several millimeters.[162]

The morphology of astroctyes is different in the CNS of mammals and lower vertebrates. Orthogonal arrays of small intramembrane particles are more prevalent in mammalian astrocytes than in amphibian astrocytes, both in intact and injured nerves.[164] The microglial cells of the CNS, responsible for disposing of dead cells and cellular debris produced by the initial trauma, differ from the Schwann cells. The former are poorly equipped to survive the conditions existing near the lesion.[126] There are also marked differences in the size of the extracellular space in regenerative and nonregenerative systems; this space is larger in the former and therefore provides potential space for axonal growth.[165]

2. Diffusible Substances

Diffusible substances, originating from nonneuronal cells, may function in the process of

regeneration as trophic agents.[140-142,166-176] Trophic agents are defined as chemical substances that have long-term effects on metabolic activities, neurite extension, survival, and differentiation of nerve cells.[177] Among such agents, NGF is the prototype which is best characterized.[178,179] NGF affects primarily PNS neurons, e.g., the growth of peripheral sympathetic fibers.[179] However, NGF was shown to affect CNS neurons, e.g., noradrenergic fibers[180] of the mammalian CNS and optic fibers of fish.[181-183] Since the discovery of NGF, additional neurotrophic factors were subsequently isolated from different sources. Some of these factors were collected *in situ*, some were derived from tissue extracts, and others came from conditioning media.[166-176,184-193] The various factors have not yet been fully purified and characterized, but most of them have been identified as polypeptides. Among the proteinaceous factors of particular interest are the protease inhibitors[194-196] released by both glial and neuronal cells. Lately, a nonpeptidic low molecular weight component which affects maintenance of CNS neurons, identified as pyruvate, was found in glial cell-conditioning media.[197]

Most of the studies with neurotrophic factors have been carried out in vitro. *In situ* experiments revealed, for example, that heart-conditioned media affected the growth of cholinergic fibers into an iris which had been implanted into the hippocampus[198] and that factors derived from goldfish brain and applied intraocularly to an eye connected to an injured optic nerve of fish accelerated the regenerative response of the corresponding retina.[199]

Axonal injury causes changes in the activity of diffusible substances originating from the environment or from the target organ. Thus, the growth of the proximal stump of a transected PNS nerve is facilitated by diffusible proteinaceous molecules possibly anchored in the basal lamina and released from the distal stump of the transected nerve.[175] Similarly, axonal injury induces increased neurite-promoting activity in extracts of the target organ.[200-204] Thus, for example, extracts prepared from denervated adult skeletal muscle contain an increased amount of neurotrophic activity which promotes survival of dissociated motor neurons and outgrowth of neurites from explants of spinal cord maintained in serum-free defined media.[201] This injury-induced increase in activity has also been observed in the brain. For example, the activity of a diffusible substance which is collected from the site of a brain lesion and which affects the survival of chick sensory neurons in culture is increased with time after the lesion.[142,173,203] Injury-induced increased activity is also manifested by the better survival of brain grafts in wound cavities several days after the injury.[202,203] In the visual system of lower and higher vertebrates, a correlation exists between the growth state of the neuron and the activity of diffusible molecules derived from it.

In both the visual system of fish and the sciatic nerve of mammals, two regenerative systems, injury causes changes in the appearance of nonneuronal-derived polypeptides.[205-208] Injury of the optic nerves of fish causes changes in the type and amount of diffusible substances originating from the nonneuronal cells surrounding the injured nerves. The changes are circumstantially correlated with the increased ability of these diffusible substances, when implanted, to trigger a regenerative response in a nonregenerative system. Thus, diffusible substances in the form of medium conditioned by regenerating optic nerves of fish are more active than intact nerves in triggering a regenerative response after being applied to injured optic nerves of adult rabbits (a nonregenerative system).[81] Media conditioned by nerve segments of neurons which are deficient with respect to their ability to regenerate lack this regeneration-supportive activity. This lack of activity may be a cause of the poor regeneration ability of the nerves. During ontogeny, however, optic neurons do possess such an activity.[82] The factors responsible for this activity were designated as growth-associated triggering factors (GATFs).[82] A similar correlation between the regenerative stage and the ability of media conditioned by the corresponding nerves to produce active substances was found in vitro with respect to the ability of the conditioned media to promote neurite outgrowth from dissociated embryonic rat cerebrocortical and hindbrain neurons.[209] In the

fish visual system, injury was also found to cause an increase in target-derived activity manifested by the promotion of glial proliferation in vitro.[210] Thus, while brain of intact fish optic nerves contains peptides which accelerate the growth of glial cells,[211] injury causes an increase in such activity.[210] Again a similarity was observed between the lower vertebrate-derived factors and those appearing in mammals.

It appears that the growing nerves have both the ability to provide triggering activities and the machinery to respond to them. The increased activity after injury in a regenerative system could evolve from an increased production and release of these molecules, the activation of preexisting molecules, or the deactivation of or a reduction in substances which inhibit the activity. For reasons not yet understood, the nonneuronal cells of the CNS of mammals fail to undergo the appropriate changes to acquire a "reactive state"[146] leading to such an activity.

3. Extracellular Matrix

The extracellular matrix plays an important role in development and regeneration.[212] During development, components of the extracellular matrix participate in neural cell migration.[213,216] The intact extracellular matrix and the availability of some of its components, such as laminin and fibronectin, are important for growth and elongation of nerve fibers both in vitro[215-217] and in vivo.[218-220] Several examples are given below. In the PNS, the existence of an intact basal lamina is a prerequisite for nerve regeneration.[218] In cases where the Schwann cells have been previously destroyed, regeneration proceeds on cues from the empty nerve tract. Regeneration in transected sciatic nerve is significantly faster when exposed to nerve guide lumens filled with laminin.[219] Proper modification of the composition of the fibrin matrix formation enhances nerve regeneration between the two stumps of transected sciatic nerves.[220] Growth of fibers from regenerating retina in vitro is supported by laminin if included in the substratum,[217] as was shown above for PNS. *In situ*, regeneration of the optic nerve is accompanied by the appearance of laminin immunoreactive sites[217] and of fibrous collagen adjacent to the basal lamina of astrocytes.[221] The role of this fibrous collagen may be similar to that of the collagen in regenerating peripheral nerves.[132-134]

In the PNS, the various components of the extracellular matrix, such as collagen and laminin, are produced by the Schwann cells.[222-227] It appears that not only PNS neurons but also CNS neurons are able to respond to laminin.[228] However, the supportive glial cells in the CNS, in contrast to their counterpart Schwann cells in the PNS or glial cells of CNS of fish, are not able to continuously provide laminin. The continuous expression of laminin may be a prerequisite for axonal growth and regeneration.[229] It seems, however, that the lack of production of laminin in the adult CNS does not evolve from intrinsic deficiency of the astrocytes, but more likely from their inappropriate activation. In vivo and in vitro studies suggest that astrocytes have the potential to produce laminin, as there is an increase in laminin appearance after transection of the rat spinal cord or optic nerve of rat.[230,231]

It therefore appears that production of laminin, at least in the CNS, depends on external stimuli. For production of laminin or other components of the extracellular matrix, a contact with the axon is a prerequisite;[222-224] some components are produced independently of such a contact.[222] It is suggested that components of the extracellular matrix provide a way to recruit trophic factors and make them accessible to the tip of the growing fibers, thereby supporting elongation.

Based on the data summarized above, it is suggested that the inability of the injury-induced proliferating glial cells to acquire a reactive state and thus to provide soluble components, extracellular matrix components, or both may be a reason for their inability to regenerate.

B. Neuronal Effects on the Environment

The nonneuronal cells which exert their activity on the neuron are themselves affected

by the neurons. As mentioned above, the expression of some properties of the nonneuronal cells depends on contact with the axons. Membranal components of the neuron (including the growth cone) and soluble materials derived from it were shown to have an effect on proliferation and differentiation (e.g., expression of S100, a glial characteristic protein, and production of myelin) of Schwann and glial cells.[232-238] The behavior acquired by the glial or Schwann cells (e.g., myelin production) depends on the type of axon with which they will come in contact because there are variations in mitogenicity of the various axolemmal preparations.[232] Neurons isolated from the CNS can selectively stimulate the proliferation of homologous nonneuronal cells without any restriction as to the brain regions from which the latter are derived.[233,234] Results of experiments in which the proximal stump of a myelinated nerve was anastomosed in a tube to the distal stump of unmyelinated nerve indicate the increased incidence of myelin fibers in the distal stump, thereby suggesting that myelinated fibers are able to establish a normal relationship with Schwann cells and become myelinated within stumps of unmyelinated fibers. This further supports the notion that axons determine the myelin production by the Schwann cells.[236] This type of relationship exists also in neurons and glia of heterologous sources, i.e., CNS glial cells can be activated by PNS axons.[238]

Based on these observations, it is possible to assume that the neuron controls the behavior of the nonneuronal cells in the resting state as well as after injury. The resting state may be mediated by neuron-derived inhibitory components (soluble or membrane associated). After injury, the proliferative response may be due in part to the elimination of these inhibitors or to the acquisition of neuronal surface molecules, presumably mitogens.

IV. INVOLVEMENT OF THE TARGET IN THE PROCESS OF REGENERATION

In order to regenerate, the neuronal fibers have to reconnect with the target organ. In the golfish, the injury-induced outgrowth results in restoration of the correct connection between the retina and the optic tectum.[12] Recent morphological studies using proline autoradiography and horseradish peroxidase staining revealed that the pathway of regenerating optic fibers is less ordered than the intact pathways.[239]

The nerve fibers and the target have a mutual relationship which includes trophic, tropic, and cell-cell interactions. The involvement of the target in regeneration has been studied mostly in the PNS. Such studies carried out in chick embryo and amphibia demonstrate that the size of the field of innervation determines the extent of development of the corresponding spinal sensory and motor neurons.[240-246] Degeneration or death of neurons during development primarily adjusts the magnitude of each neuronal population to the size or the functional needs of its projection field.[245]

The specificity of the influence of the target tissue is demonstrated by transplantation experiments that involve the transplantation of fetal brain tissue into various brain regions. The preferred location for survival and growth of the transplanted neurons is adjacent to the original target organ.[247-250] These experiments include grafts of fetal brain regions into adult and developing host brains, and they may indicate that the environment of mature CNS neurons, although not supportive of growth of mature CNS axons, may in part be supportive of growth of axons from developing neurons. Embryonic retina, cortex, and tectum transplanted in adult superior colliculus rat survived and differentiated to a degree which was less than when the implantation was to newborn.[248] The gestation age of the donor of the implants of embryonic CNS determines its survival in the host and its degree of differentiation.[247] Topographical preference was also observed in cerebellar pieces transplanted into the tectum of goldfish. The cerebellar pieces survived for long periods of time but did not become innervated.[251]

Studies of the relationship between growth and differentiation of neurons and their target

have been extended with the availability of tissue-culture techniques. Cocultivation of neurons with various target tissues resulted in the growth of neurons directly to the physiological target.[252-255] In many cases, the effect of the explanted tissue could be replaced by tissue extracts or by media conditioned by the target organs.[256-260] Proteinaceous factors, which originate from the target cells, are responsible for these activities. However, their purification and characterization have been hampered due to their low abundance within the tissue.

Tectal pieces of goldfish, cocultured with regenerating retinal pieces, were shown to enhance neuritic outgrowth from the retinas. The extent of growth was tenfold larger in comparison to the growth from cultured retinas in the absence of any tectal pieces.[261]

The promotive effect of the tectal pieces could also be achieved by soluble substances derived either from the tectal pieces or from the whole goldfish brain.[199,262] This activity is mediated by substances distinct from NGF in spite of the NGF-like activity found in the goldfish brain.[263,264]

The possible role of cell-cell interactions as a mechanism for determining specific neuron-target reconnection[12] has gained some support from studies employing monoclonal antibodies which showed the existence of topographical position markers on both retina and target cells.[265-267] The topographical-determining mechanism of the neuron-target reconnection[268-275] is beyond the scope of this paper.

V. SUMMARY

Injury of an axon leads to at least four independent events, summarized in Figure 1: first, deprivation of the nerve cell body from target-derived or mediated substances, which leads to a derepressed or a permissive state (2); second, disruption of anterograde transport, with a resultant accumulation of anterogradely transported molecules; third, environmental response with possible consequent changes in constituents of the extracellular matrix and substances secreted from the surrounding cells; and fourth, appearance of growth inhibitors and modified protease activity. It seems that the first three of these events are obligatory, but not sufficient, i.e., they lead to a growth state (3) only if the cell body is able to respond to the injury-induced signals from the environment (a and b). The regenerative state is characterized by alterations in protein synthesis and axonal transport and by sprouting activity. The subsequent elongation of the growing fibers (4) depends on a continuous supply of appropriate growth factors. These factors are presumably anchored to the appropriate extracellular matrix that serves as a substratum for elongating fibers. It should be mentioned that the proliferating nonneuronal cells have a conducive effect on regeneration by forming a scaffold for the growing fibers. Accordingly, the lack of regeneration may stem from a deficiency in the ability of glial cells to provide the appropriate soluble components or from insufficient formation of extracellular matrix. In this respect, one may consider regeneration of an injured axon as a process which involves regeneration of both the nonneuronal cells and the supported axons. The regeneration of glial cells may fulfill the rules which are applied to regeneration of any other proliferating tissue. Furthermore, the processes of regeneration in the axon and the glial cells are mutually dependent. Perhaps the triggering factors provided by the nonneuronal cells affect the nonneuronal cells themselves by modulating their postlesion gliosis and thereby inducing their appropriate activation. In such a case, regeneration of nonneuronal cells may resemble an autocrine type of regulation that exists also during ontogeny. The growth regulation is shifted back to the paracrine type upon neuronal maturation or cessation of axonal growth. When the elongating fibers reach the vicinity of the target organ, they are under the influence of the target-derived factors, which guide the fibers and eventually cease their elongation (5).

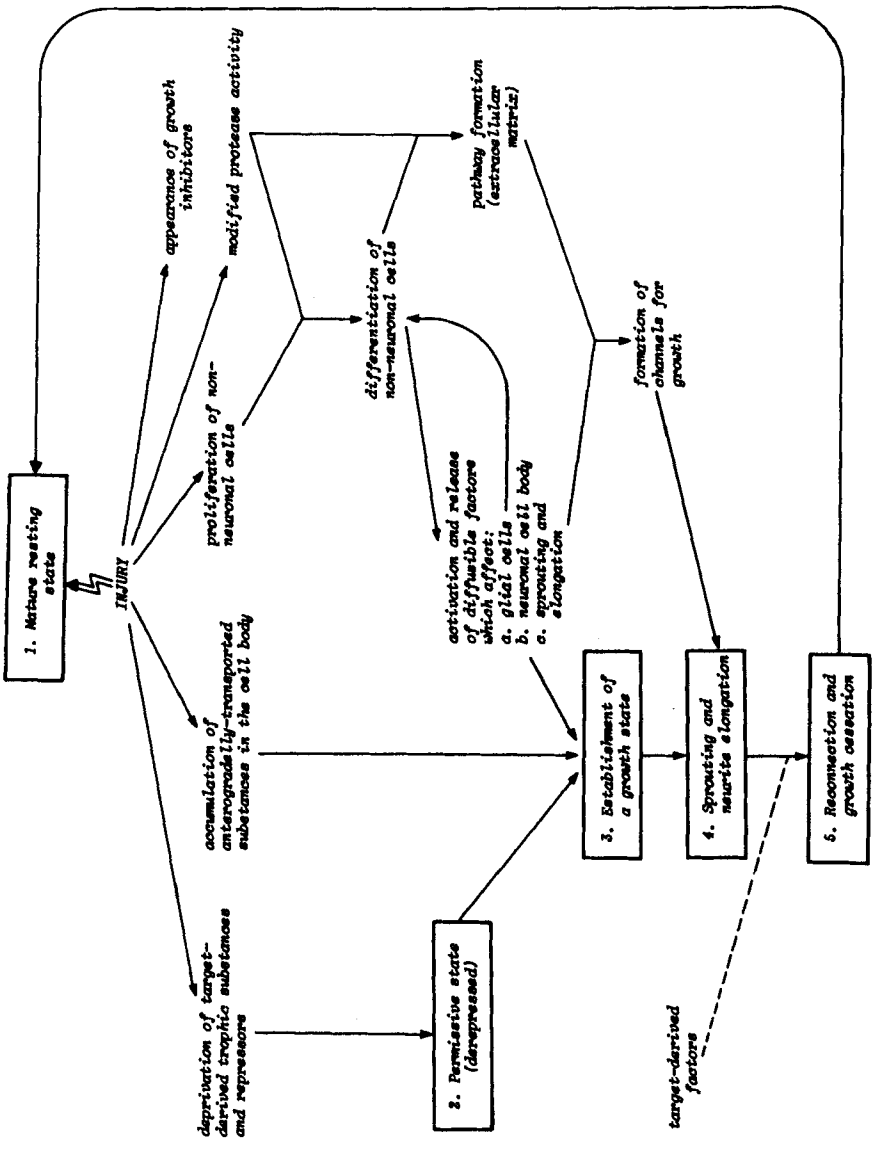

FIGURE 1. A guide for the regeneration process for the perplexed.

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