*Ann. Rev. Pharmacol. Toxicol. 1985. 25:643–66*




# THE MECHANISMS OF ACRYLAMIDE AXONOPATHY

*Matthew S. Miller and Peter S. Spencer*

Institute of Neurotoxicology and Departments of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, New York 10461

## INTRODUCTION

Degeneration of the distal axons of long and large-diameter peripheral nerve fibers is perhaps the most common of all toxic peripheral nerve disorders. Degeneration commonly spreads centrally along affected nerve tracts in a dying-back fashion (1). Axonal disorders of this type are grouped under the term *central-peripheral distal axonopathy* to emphasize the contemporaneous onset of distal retrograde axonal degeneration in long nerve-fiber tracts in both the central nervous system (CNS) and the peripheral nervous system (PNS) (2). Axonopathy refers exclusively to the primary degeneration of axons, although associated changes may occur in corresponding neuronal perikarya. Schwann cells and oligodendrocytes enveloping affected axons in the PNS and CNS, respectively, commonly undergo secondary changes, including mitosis and loss of myelin maintenance, while fibroblasts and astrocytes become involved later in the degenerative process.

Distal axonopathy can result from a single or, more commonly, repeated exposure to a variety of chemically unrelated agents, or it can occur with apparent spontaneity in a number of abnormal nutritional or metabolic states (3). Although the biochemical mechanisms underlying axonal degeneration are unknown, some progress has been made in recent years in understanding the biochemical events that precede and accompany axon degeneration in certain toxic neuropathies, notably those associated with repeated exposure to acrylamide or aliphatic γ-diketones. Multidisciplinary investigation of these neuropathies in laboratory animals and organotypic tissue cultures has revealed several previously unknown basic mechanisms that may be involved in the maintenance of the axon and the initiation and regulation of the degenerative



0362-1642/85/415-0643$02.00

process. This review focuses on the mechanisms underlying the degeneration of the axon in acrylamide neuropathy, a subject previously reviewed by Spencer & Schaumburg (4) and Le Quesne & Tilson (5, 6). Readers interested in equally important developments in understanding γ-diketone axonopathy are referred to other review articles (7, 8).

## MAINTENANCE OF THE AXON

### *Anatomic Considerations*

An understanding of the basic processes by which normal axonal integrity is maintained is essential to any consideration of toxic/metabolic perturbations that precipitate axonal degeneration. The axon is a unique cellular process that communicates electrically encoded information over long distances. No cell other than the neuron projects cellular processes over distances comparable to that of the axon. Bipolar sensory neurons, for example, their neuronal perikarya located in lumbar spinal ganglia, project a peripheral axon that may extend the entire length of a limb to terminate in the toe while simultaneously projecting a central axon to the brain stem. In the adult human, therefore, primary sensory neurons maintain axons exceeding five feet in length.

While the mammalian axon has the metabolic machinery to synthesize proteins and lipids and to modify them post-translationally by phosphorylation and other mechanisms (9), axoplasm has a negligible capacity for lipid and protein synthesis (9, 10). Some metabolic support may be provided by Schwann cells or oligodendrocytes, but in mammals this is insufficient to maintain the axon after disconnection from its neuronal perikaryon. The axon, therefore, is largely dependent on the neuronal perikaryon for its supply of metabolic requirements (11–13). This has necessitated the development of bidirectional intraneuronal transport mechanisms to shuttle materials and information between the perikaryon, the axon, and the nerve terminal.

### *Axonal Transport*

Axoplasmic transport may be divided into at least three anterograde and two retrograde transport systems (14, 15).

ANTEROGRADE AXONAL TRANSPORT Materials synthesized in the neuronal perikaryon are moved distally along the axon at various velocities: at least two slow and one fast anterograde transport systems have been recognized. Slow anterograde axoplasmic transport systems supply constituents that maintain the bulk of the axonal cytoskeleton and glycolytic function. Slow component a (SCa) transports neurofilament triplet proteins and tubulin at a rate of approximately 0.1–2 mm a day; slow component b (SCb) transports a variety of structural proteins, including actin, at a rate of 2–4 mm a day (15). Several,

perhaps all, glycolytic enzymes are transported in SCb (13). Thus, in a typical human nerve terminating in the foot, up to eight months may elapse before a glycolytic enzyme synthesized in the neuronal perikaryon arrives in the terminal axon.

Fast anterograde axoplasmic transport moves materials distally along the axon at rates of approximately 400 mm a day. This system is primarily involved in transporting membrane-associated proteins and glycoproteins for maintenance of axolemma, the axon surface membrane (16–18). Also transported by the fast anterograde axoplasmic transport system are peptides involved in neurotransmission, such as dopamine-β-hydroxylase, some forms of acetylcholinesterase, and substance P (19–21), as well as the classical neurotransmitters acetylcholine and norepinephrine (14). Fast anterograde axoplasmic transport therefore appears to be involved in the movement of substances required for the maintenance of axon membranes and for neurotransmission.



RETROGRADE AXONAL TRANSPORT This transport system is the least understood component of neuronal transport. The system probably functions to communicate information on the state of the axon to the neuronal perikaryon, as well as to allow the return of certain axoplasmic constituents for reprocessing in the perikaryon (22–27).

The retrograde system translocates a variety of substances from the periphery to the soma at a rate of approximately 150–250 mm a day [reviewed in (14)]. Substances transported by the retrograde system include acetylcholinesterase, adrenergic granules, lysosomes, and fucosyl glycoproteins (28–30). In addition, a variety of endogenous or foreign substances is taken up nonspecifically by axon terminals and may utilize the retrograde transport system: these include serum albumin and horseradish peroxidase (31, 32). Nerve Growth Factor, a protein thought to act as a trophic substance in some neurons (33–35), and tetanus toxin (31, 36) interact with membrane-binding sites located on the distal terminals of neurons, undergo pinocytosis, and retrograde axon transport. The transport of Nerve Growth Factor appears to be limited to sensory and adrenergic neurons, while tetanus toxin is transported without apparent neuronal specificity (34).

A slow retrograde axoplasmic transport system also has been described (37). Transport occurs at a rate of approximately 3–6 mm a day and a single protein, thought to be albumin, is involved.

THE GRADIENT HYPOTHESIS Proteins are probably utilized and/or degraded while undergoing anterograde axonal transport. In theory, this creates a gradient of material in which the concentration of anterogradely transported materials is greater in proximal than in distal portions of axons. Gradients of this nature have been reported in peripheral nerves for phosphoprotein, ace-

tylcholinesterase, choline acetylase, and non-specific carboxyesterases (38). Thus, little or no surplus of axonally transported proteins may be available in distal aspects of long axons. This may render distal axons vulnerable to toxins that diminish the activity or availability of enzymes or other important proteins delivered to axoplasm by axonal transport.

## ACRYLAMIDE NEUROPATHY

### *Introduction*

Changes in axonal transport or the availability of essential materials may be essential elements of the mechanism underlying the distal retrograde axonal degeneration found in humans and animals repeatedly exposed by a systemic route to acrylamide ($CH_2 = CHCONH_2$). Although the critical biochemical events leading to axonopathy remain to be determined, acrylamide is known to react spontaneously with hydroxyl-, amino-, and sulfhydryl-containing compounds (39–44).

### *Neuropathy in Humans*

As early as the 1950s, researchers realized that industrial workers exposed to monomeric acrylamide are at risk for peripheral neuropathy, a disorder characterized by a stocking-and-glove distribution of sensory, motor, and autonomic deficits and accompanied by excessive tiredness and ataxia (45, 46). Subjects present with cold, blue hands and commonly report unsteadiness, muscle weakness, paresthesia, and numbness in the hands and/or feet. Tendon reflexes disappear, probably in association with degenerative changes in muscle-spindle afferents (47, 48). Position sense in distal joints is commonly, but not always, lost (46). Vibration sense is lost distally, but temperature, pressure, and other objective sensory modalities remain intact (45). Recovery from mild forms of acrylamide neuropathy is usually complete, normally occurring within a few months (45, 46, 49). However, severely affected patients may never totally recover, experiencing residual ataxia, distal weakness, and sensory disturbances (50).

### *Experimental Neuropathy*

Repeated exposure to acrylamide has been shown to produce neuropathy in cats (51–53), rats (53–56), mice (57–59), guinea pigs, rabbits, monkeys (53, 60), chickens, and goldfish (61). As with humans, laboratory animals also develop ataxia and weakness most apparent in the hind limbs.

### *The Disposition of Acrylamide*

Numerous studies have attempted to elucidate the mechanism underlying the neurotoxic property of acrylamide. Kuperman (51) found the effects of acryla-

mide in the cat to be independent of route of administration or duration of intoxication, the appearance of hindlimb weakness first occurring when an apparent cumulative dose threshold had been exceeded. He believed that either acrylamide or a metabolite accumulated at the target site or the substance impaired a slowly resolving process. Functional impairment would result when a critical threshold was surpassed. While recent studies have challenged the linearity of the relationship between a cumulative dose of acrylamide and the onset of neuropathy, there is little doubt that acrylamide has cumulative effects. Thus, studies addressing the disposition of acrylamide have become essential to understanding the basis of acrylamide-induced neuropathy.

Following administration, acrylamide is rapidly distributed to all tissues, metabolized, and excreted (61–63). The distribution and elimination kinetics for acrylamide are influenced only slightly by the route of administration. Using a single dose of 2,3-$^{14}$C-acrylamide, Miller et al (63) demonstrated equivalent concentrations of radiolabel in all tissues except erythrocytes. Neural tissue accumulated less than 1% of the dose of acrylamide. Tissue radiolabel content decayed in a bi-exponential fashion ($t_{1/2}$ approximately equal to 5 hours). Only erythrocytes demonstrated significant retention of radiolabel (63, 64). The elimination constants for parent acrylamide ($t_{1/2}$ approximately equal to 2 hours) are faster than those for total radioactivity in all tissues. Thus, acrylamide itself is not selectively retained or concentrated in neural tissues following single (63) or repeated dosing (65). A small percentage of radiolabel persists in all tissues for several weeks ($t_{1/2}$ approximately equal to 8 days). Tissue-associated radiolabel has been reported to be protein-bound, but the possibility that metabolic fragments of acrylamide are incorporated into protein via normal protein synthetic mechanisms has not been excluded. This notion gains support from the observation that $^{14}CO_2$ is expired by laboratory animals treated with $^{14}$C-acrylamide (44).

The major route of biotransformation of acrylamide is conjugation with the tripeptide glutathione (62, 63, 66). This route appears detoxifying, since depletion of tissue non-protein sulfhydryl content increases the neurotoxic potency of acrylamide (67). Acrylamide enzymatically and non-enzymatically reacts with glutathione (61–63, 67, 68) and is eventually excreted in urine as N-acetyl-S-(3-amino-3-oxypropyl)-cysteine (63, 66). Thus, it appears that neuropathy is mediated by a direct action of parent acrylamide and that conjugation of acrylamide with glutathione is a detoxification process. Acrylamide is capable of inhibiting the enzyme activity of glutathione-S-transferase both in vitro and in vivo (67–72). This suggests that acrylamide may inhibit its own detoxification along the glutathione conjugation pathway, although this has yet to be demonstrated directly.

In addition to conjugation with glutathione, acrylamide appears to undergo biotransformation mediated by the microsomal cytochrome P-450 system.

Kaplan et al (73) reported that free acrylamide disappears faster in liver homogenates prepared from animals whose microsomal cytochrome P-450 levels have been elevated by pretreatment with phenobarbitol. Further study revealed that pretreatment of rats with microsomal-inducing agents (phenobarbitol or dithiothreitol) may delay the onset of acrylamide-induced neuropathy (73, 74). However, this finding has not been confirmed by other investigators (61, 62, 65).

## *Structural Analogs of Acrylamide*

Several structural analogs of acrylamide have been evaluated for neurotoxic potential in an attempt to determine which portion of the acrylamide molecule is involved in the induction of neuropathy (44, 61, 62, 74). Acrylamide analogs that produce neuropathy include N-isopropylacrylamide, N-methylacrylamide, methacrylamide, N-hydroxymethylacrylamide, and N,N-diethylacrylamide (74). None of these compounds demonstrates greater neurotoxic potency than acrylamide. Positive analogs (except methacrylamide) differ from acrylamide in the substitution of the amide group, although other amide-substituted analogs, such as methylene(bis)acrylamide and N,N-pentamethylene acrylamide, appear unable to produce neuropathy in laboratory animals. Reduction of the double bond of acrylamide, or deletion of the nitrogen atom, eliminates the potential of the compound to induce neuropathy. Thus, it appears that the acrylyl moiety ($CH_2CHCO-$) of acrylamide is essential for neurotoxic potential, while amide substitution can profoundly modify neurotoxic potential.

Acrylamide's high affinity for sulfhydryl nucleophiles has been considered a potential means by which the substance could inactivate critical axonal proteins (44, 75–82). However, no clear relationship exists between in vitro sulfhydryl reactivity and the potential to produce neuropathy, since N,N'-diethylacrylamide and N-methylacrylamide, compounds much less reactive with sulfhydryl groups than acrylamide (44), prove to be neurotoxic (61, 62). Thus, while it is possible that reaction with sulfhydryl-dependent proteins may be involved in the pathogenesis of acrylamide neuropathy, it appears that reaction with sulfhydryl moieties is not the sole mechanism by which acrylamide produces this disorder.

## *Effects on Axon Structure*

Fullerton & Barnes (54) were the first to demonstrate the involvement of peripheral nerves in acrylamide neurotoxicity. By comparing the size distribution of myelinated fibers in nerves from acrylamide-treated and untreated rats, they were able to demonstrate a reduction in the number of large-diameter fibers in cross sections of hindlimb nerves from treated animals that revealed various stages of nerve-fiber degeneration (Figure 1). Spencer & Schaumburg

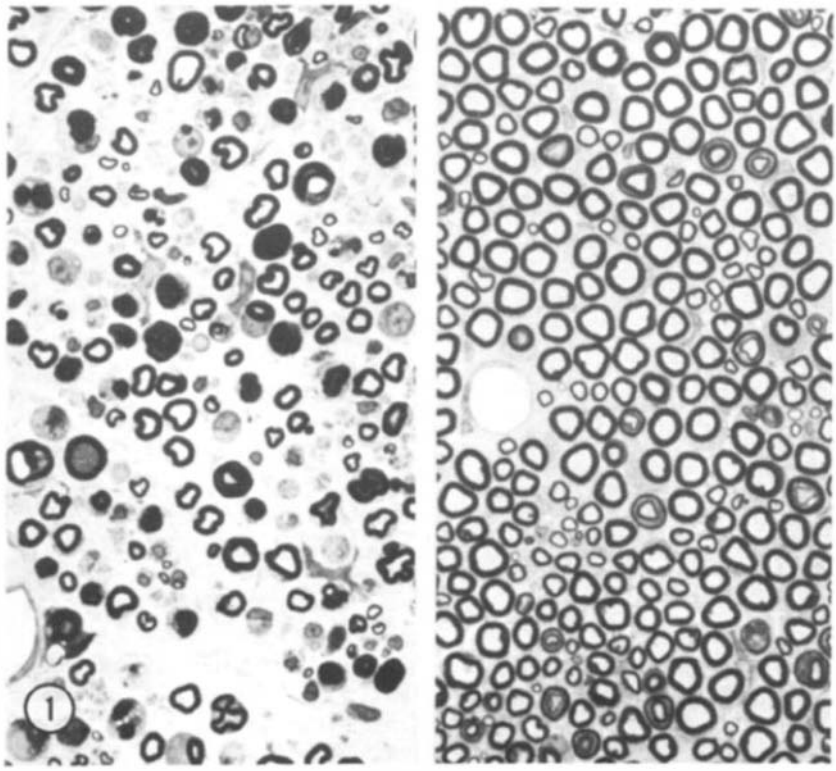


*Figure 1* Left: Degeneration of myelinated fibers in the tibial nerve of a rat treated with 0.35% acrylamide in diet for 28 days. Right: Normal tibial nerve from an age-matched control rat. One-micrometer epoxy cross-sections stained with toluidine blue × 500.

(2) demonstrated the spatial-temporal evolution of retrograde degeneration in rats treated with acrylamide and showed that long nerve fibers both in peripheral nerves and in ascending tracts of the spinal cord are also involved in the dying-back process (Figure 2).

Prineas (83) first reported the ultrastructural features of axonal degeneration. Initial abnormalities include the presence of abnormally large numbers of intermediate filaments, mitochondria, and other organelles. Some of the latter appear to be sequestered and selectively removed by invaginations of adaxonal cytoplasm of the Schwann cell or oligodendrocyte (84). Shortly before the axon breaks down, there is a loss of neurotubules and neurofilaments and dissolution of the axolemma.

Schaumburg and associates (48) demonstrated that the earliest detectable morphological change produced by acrylamide in the cat occurs in the Pacinian corpuscles of the toe pads. Reduction of the generator potential of the corpuscle

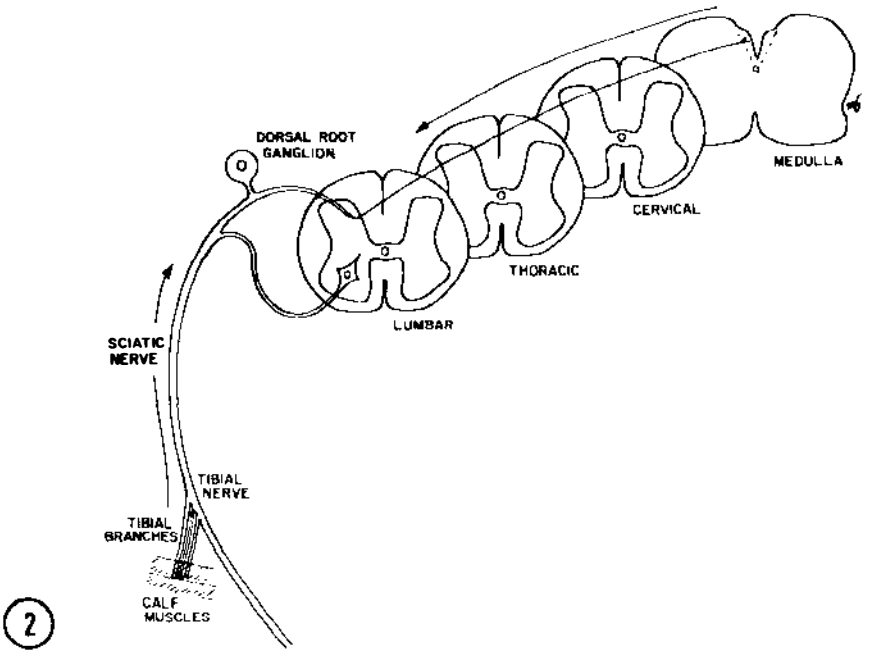


*Figure 2* Diagrammatical sketch showing the spatial-temporal distribution of myelinated nerve-fiber degeneration in animals treated with acrylamide. Changes first appear in tibial branches supplying the calf musculature and in the termination of the gracile tract in the medulla oblongata. Arrows depict the temporal progression of degeneration along affected nerve-fiber pathways.

axon precedes the first structural damage produced by acrylamide, namely, loss of filopod axon processes containing microfilaments (4, 85). Sequential degeneration of adjacent primary annulospiral endings of muscle spindles, secondary muscle spindle endings, and motor nerve terminals then occurs. Degeneration is associated with accumulations of vesicles and neurofilaments in the preterminal region of the axon. This suggests that distal axonopathies, such as acrylamide neuropathy, involve a biochemical lesion that may lead to alterations in axoplasmic transport (83, 86).

## *Effects on Axonal Transport*

Numerous studies have examined the effects of acrylamide on anterograde axonal transport in experimental animals at times when functional signs of neuropathy are evident and distal axons are undergoing degeneration (86–90). The studies of Sidenius & Jakobsen (91), Sumner and colleagues (92), and Griffin & Price (93) revealed either no change or slight decreases (10–15%) in the rate of fast anterograde axonal transport, with no detectable change in slow anterograde transport in association with acrylamide-induced neuropathy.

However, studies by Weir et al (88) and Souyri et al (89) suggest that fast anterograde transport of protein is altered by acrylamide. Furthermore, analysis of acetylcholinesterase transport in acrylamide-intoxicated chickens revealed a 60% reduction in the rapidly transported A12 form of acetylcholinesterase, while slowly transported forms (G1 and G2) were unaffected by acrylamide (94).

Caution is appropriate in the interpretation of these studies. First, it is unclear whether the reported abnormalities are associated with the cause or the effect of ultrastructural abnormalities induced by acrylamide. A second possibility is that changes in transport may occur because acrylamide impairs protein synthesis (95, 96), a property shared by acrylamide analogs that lack the ability to induce neuropathy in laboratory animals. The third important consideration is that measured changes in anterograde transport in proximal axons may differ from those occurring in distal axons.

Examination of the effect of acrylamide on bidirectional fast anterograde axoplasmic transport of radiolabeled protein revealed a large deficit in retrograde fast transport in animals that displayed ataxia and hindlimb weakness (97). Subsequent studies by Sidenius & Jakobsen (91) showed that retrograde transport is dramatically affected in acrylamide-intoxicated animals (98). The defect is characterized by a reduction in the quantity of material undergoing retrograde transport. Additionally, evaluation of retrograde axonal transport after varying doses of acrylamide revealed that alterations in retrograde transport *preceded* the development of functional signs of neuropathy (98–100). By contrast, N,N'-methylene(bis)acrylamide and N-hydroxymethylacrylamide, two acrylamide analogs that do not produce neuropathy in the doses employed, fail to affect retrograde axonal transport of protein, thereby suggesting a specific association between axonopathy and defects in retrograde transport. More recently, it has been demonstrated that acrylamide administration inhibits retrograde axonal transport but not neuronal uptake of horseradish peroxidase (HRP)(101), and that *single* doses of acrylamide produce marked deficits in the rate of retrograde axonal transport of radiolabeled Nerve Growth Factor (99) and tetanus toxin (100).

The actions of single doses of acrylamide on retrograde axonal transport have been investigated in an attempt to distinguish between the effects on axonal transport that result from the direct action of acrylamide and those that occur secondarily to axonal changes induced by repeated acrylamide dosing. Single doses of acrylamide, similar to those used for repeat-dosing studies, reduce the rate of retrograde axonal transport in a dose-dependent manner (Figure 3). Retrograde transport in sensory neurons appears more vulnerable to the actions of acrylamide than motor neurons. These data correlate well with previous electrophysiologic (47) and morphologic (48) studies demonstrating the increased susceptibility of sensory axons to acrylamide. The extent to which

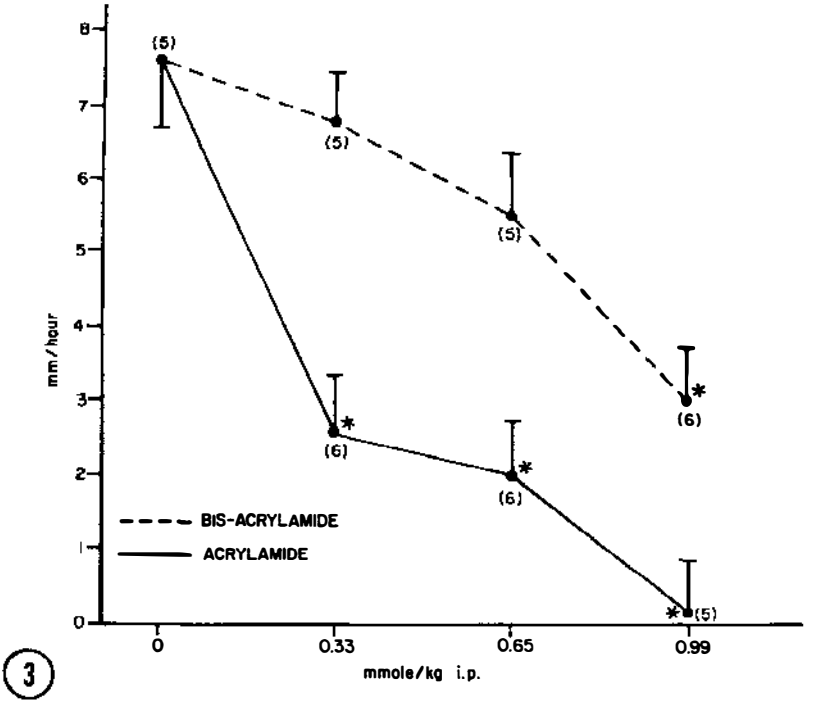


*Figure 3* Effect of acrylamide (————) and N,N'-methylene-bis-acrylamide (— — — —) on rate of retrograde transport of $^{125}$I-tetanus toxin. Values are mean ± SEM. Number of animals per group is in parenthesis. *p less than 0.05 versus saline-injected control value by analysis of variance and Scheffe's test. Reprinted from (100) with permission.

retrograde axonal transport is affected by acrylamide increases with repeated acrylamide administration and precedes the appearance of ataxia and weakness (Figure 4).

A recent study has examined the effects of single doses of acrylamide on slow anterograde axoplasmic transport (102). Data revealed a modest, non-specific defect in slow anterograde axonal transport of all proteins visible on fluorographs. However, the possibility that acrylamide-induced alterations in protein synthesis are responsible for the apparent slow transport defect cannot as yet be excluded.

In summary, profound changes in axonal transport are produced by single doses of acrylamide that, if repeated over the course of a few weeks, precipitate functional signs of neuropathy. These transport defects precede by many days the onset of functional or morphologic signs of axonal compromise. Further investigation is required to determine how these early changes in axonal

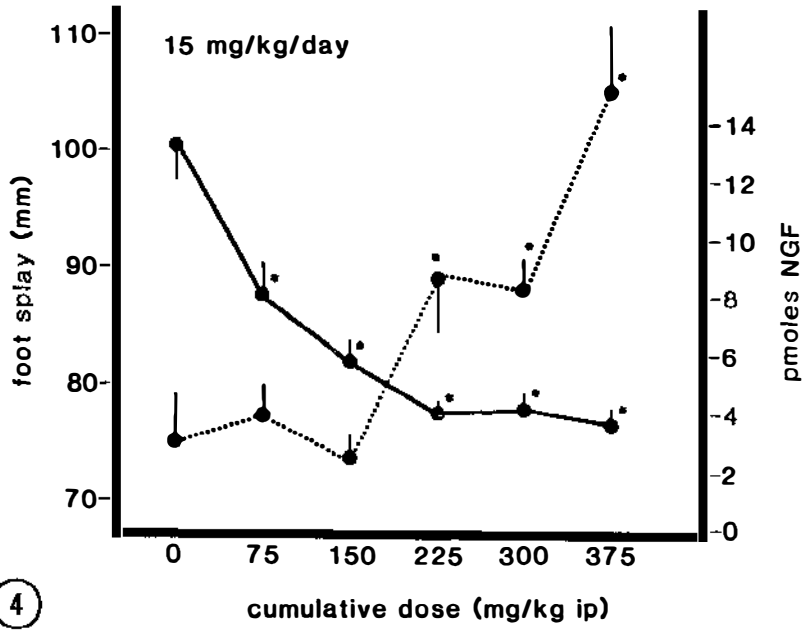


*Figure 4* Effect of repeated administration of acrylamide (15 mg/kg/day, intraperitoneally) on the retrograde axonal transport of nerve growth factor (NGF)(————) and hindlimb footsplay (— — — —). Retrograde axonal transport was assessed by determining the quantity of $^{125}$I-NGF transport to dorsal root ganglia following peripheral injection. *p less than 0.05 versus time 0 value by analysis of variance and Scheffe's test. Reprinted from (99) with permission.

transport are related to the primary biochemical lesion(s) and the subsequent axonal demise.

## *The Significance of Retrograde Transport Defects*

Retrograde axonal transport may be instrumental in the initiation of regenerative and repair processes following neuronal injury (22). Several studies (22–27) have supported the concept that changes in retrograde transport may be one mechanism by which perikaryal responses to peripheral axon lesions are initiated. Bisby & Bulgar (22) observed that perikaryal proteins labeled with $^{3}$H-leucine are transported by fast anterograde axoplasmic transport to the distal ends of both sensory and motor neurons. The direction of transport is then reversed and the radioactivity is transported in a retrograde fashion. Axonal injury by ligation causes materials transported by the fast anterograde axoplasmic transport system to turn around proximal to the ligation. These findings have led to the hypothesis that materials returning from the peripheral process via the retrograde transport system may serve to inform the soma of the

integrity of the peripheral process and may be intimately involved in the initiation of the regenerative process. Recent experiments by Singer et al (27) have demonstrated that both metabolic and morphologic changes in the perikarya following nerve transection may be delayed by administration of colchicine to the nerve trunk, a compound known to effect bidirectional axonal transport blockade (103–108). These data suggest that it may be the appearance of a signal, rather than the lack of one, that is responsible for initiating perikaryal responses to axon damage.

The observation that single doses of acrylamide slow retrograde transport (99, 100) has led to the hypothesis that distal axonopathy may be the result of a failure to inform the soma of the need to repair nonspecific axonal lesions. This hypothesis is supported by previous studies (109, 110) demonstrating that acrylamide significantly slows regenerative terminal sprouting, possibly as a result of interactions with sulfhydryl groups (78, 79), while fast anterograde transport of protein remains normal (110). Further support for this hypothesis is found in the studies of Cavanagh & Gysbers (111) in which the administration of acrylamide was found to potentiate retrograde degeneration of nerve fibers in the proximal stump of a severed nerve.

Taken in concert, these data suggest that acrylamide may prevent the neuronal perikaryon from responding normally to axonal transection. Several studies have suggested that repair-specific proteins are synthesized in neuronal perikarya supporting regenerating axons (11, 12, 112–116). Many of these growth-associated proteins (GAPs) are found only in regenerating axons, suggesting that they appear as a consequence of qualitative alterations in gene expression and that the synthesis of these proteins is probably dependent on transcription of new mRNA species (117–120). Whether acrylamide administration prevents the synthesis of GAPs is currently unknown. However, recent studies demonstrate that single and repeated doses of acrylamide inhibit the induction of perikaryal ornithine decarboxylase (ODC, EC 4.1.1.17) activity and the rate of total RNA synthesis in response to axon damage (121), possibly by blocking the retrograde axonal transport of putative repair-initiating factor(s) (100). ODC is the rate-limiting enzyme in polyamine synthesis (122) and represents an extremely sensitive means by which perikaryal responses to peripheral nerve damage may be quantified. Polyamines are required for a variety of hyperplastic and hypertrophic cellular processes, including axonal differentiation, maturation, and regeneration (123–128). ODC has a half-life of approximately 11–15 minutes (129) and thus must be continually synthesized. Synthesis of ODC appears to be regulated predominately at the level of transcription because actinomycin D readily blocks its induction in neuronal perikarya. Although a recent study has demonstrated that induction of ODC activity may not be a prerequisite for regeneration of goldfish optic nerve (130, 131), it is apparent that ODC activity is a useful marker of alterations in

DNA-dependent transcription associated with cell proliferation, growth, or regeneration. That acrylamide inhibits the induction of ODC activity and RNA synthesis following nerve transsection supports the hypothesis that acrylamide attenuates perikaryal responses to axon damage.

## *Changes in Neuronal Perikarya*

In addition to a possible action of acrylamide on retrograde transport–mediated perikaryal repair responses, it appears likely that acrylamide may have a direct action on the perikaryon itself. Morphologic evaluation of lumbar spinal ganglia following acrylamide administration has revealed cytoplasmic reorganization with nuclear and Nissl changes resembling chromatolysis (132–136). Chromatolysis is a poorly understood stereotypic morphologic reorganization of the perikaryon following axon transsection or injury. The intensity of the chromatolytic response increases with more proximal axon lesions and is rarely associated with distal toxic neuropathies [(83; reviewed in 137)]. Although the significance of the chromatolytic-like response in spinal ganglia following acrylamide administration is unclear, it suggests that the actions of acrylamide may in some way mimic axon transsection, possibly as a result of retrograde axonal transport blockade. It has also been suggested that acrylamide might produce distal axonopathy by disturbing the metabolic integrity of neuronal perikarya (132–135), although the observation that characteristic pathological changes appear in axons locally exposed to acrylamide (and other agents that induce axonopathy) has been taken as evidence that acrylamide is a direct axonal toxin (138).

## *The Mechanism of Altered Axonal Transport*

ACTIONS ON TUBULIN Bidirectional fast axonal transport is blocked by agents that bind to tubulin (103–108). Colchicine specifically binds to tubulin, resulting in the disassembly of labile microtubules, including those present in axoplasm. Similar effects are produced by the *Vinca* alkaloids, vinblastine, vincristine, and vindesine (an amide derivative), a more potent class of tubulin-binding agents. Ultrastructural studies demonstrate a rapid loss or numerical reduction of axonal microtubules following local application of tubulin-binding agents to peripheral nerves. In addition to microtubules, it has been suggested that smooth endoplasmic reticulum and neurofilaments may be important structural components of the axonal transport mechanism. No evidence for the morphologic alteration of any of the putative structural components of axonal transport, microtubules, neurofilaments, or smooth endoplasmic reticulum, is apparent in the sciatic nerve of animals treated with single doses of acrylamide that decrease rates of retrograde axonal transport (M. S. Miller, P. S. Spencer, unpublished data). Thus, it appears that acrylamide initially alters axonal

transport through a biochemical lesion without obvious disruption of structural axoplasmic components.

A recent study, however, has demonstrated profound decreases in the binding of $^{3}$H-colchicine to tubulin in sciatic nerve and spinal cord, but not in cerebellum or brain, following prolonged exposure to acrylamide (139). While these data might suggest that acrylamide alters existing tubulin or decreases tissue tubulin content, the time-course for the depletion of $^{3}$H-colchicine binding by acrylamide appears to parallel the appearance of degenerating axons in peripheral nerves and spinal cord. Thus, it appears likely that alterations in $^{3}$H-colchicine binding reflect decreases in tubulin content associated with axonal degeneration rather than specific alterations in tubulin itself. However, a direct action of acrylamide on tubulin and microtubules cannot be excluded at this time.

ACTIONS ON HIGH-ENERGY PHOSPHATE PRODUCTION Maintenance of bidirectional fast axonal transport is clearly dependent on the constant availability of adequate supplies of high-energy phosphate [reviewed in (137)]. Transport is impaired by the specific inhibition of the metabolic steps in glycolysis, the Kreb's cycle, electron transfer chain, or terminal phosphorylation. Inhibition of axonal transport occurs when high-energy phosphate (ATP and creatine phosphate) declines to approximately half of levels found in normal mammalian nerves (140). The actual critical threshold value of axonal high-energy phosphate required to maintain fast axoplasmic transport is unknown because it is technically impossible to distinguish levels of high-energy phosphate compounds in the axon from those in Schwann cells or oligodendrocytes.

Since the enzymes involved in the production of high-energy phosphate compounds (enzymes of glycolysis and oxidative phosphorylation) undergo axonal transport, it is possible that small decrements in axonal energy production could result in dramatic deficits in the distal axon. The transport of enzymes involved in energy transformation from the neuron perikayon to the distal axon may be slowed by locally diminished high-energy phosphate supplies. Were this to occur, the failure to transport required enzymes would further compromise high-energy phosphate production, which would in turn further inhibit axonal transport. This cascade of events would result in a proximal-to-distal gradient in energy transformation, with levels in distal axons perhaps falling below those required to maintain axonal transport and, therefore, maintenance of axonal integrity more distally. Thus, a potential mechanism by which acrylamide may alter axoplasmic transport involves interference with enzymatic processes associated with the production, storage, or utilization of high-energy phosphate compounds.

## *Effects on Glycolytic Enzymes*

Acrylamide is known to be highly reactive with cellular sulfhydryl groups (39–44) and reacts spontaneously with the non-protein sulfhydryl compound glutathione (61–63). It has been suggested that alterations in the activity of sulfhydryl-dependent glycolytic enzymes may be the critical biochemical lesion underlying acrylamide-induced distal axonopathy (76, 77, 141). Unlike other cell types, neurons with long axons would be slow to replace glycolytic enzymes irreversibly inhibited by reaction with acrylamide due to the large anatomic separation between perikaryal sites of protein synthesis and distal axoplasm. When added to purified enzyme or rat brain homogenate, acrylamide inhibits the activity of phosphofructokinase (PFK), enolase, neuron-specific enolase (NSE), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), but not lactic acid dehydrogenase (76, 77, 80–82, 142). The inhibition of glycolytic enzyme activity by acrylamide is irreversible and may be attenuated by preincubation with the sulfhydryl-protecting compound dithiothreitol. Thus, inhibition of glycolytic enzyme activity by acrylamide appears to be the result of a covalent interaction with critical enzyme sulfhydryl moieties. While inhibition of PFK and GAPDH activity by acrylamide is irreversible, the effect of acrylamide on enolase is reversed by dialysis (76, 77). This suggests that the covalent interaction of acrylamide with enolase sulfhydryl moieties is not the mechanism by which acrylamide inhibits enolase activity in peripheral nerve. Following the repeated administration of acrylamide to laboratory animals, the enzyme activities of GAPDH and NSE in peripheral nerve are reduced by approximately 35% (82). The activity of LDH or the rate-limiting glycolytic enzyme PFK remain unchanged (77). These findings raise the possibility that acrylamide alters axonal glycolysis and energy production, although it is presently impossible to determine whether changes in enzyme activity are occurring within the axon and/or the Schwann cell compartment.

Although the activity of GAPDH and NSE is diminished by acrylamide, neither enzyme is normally thought to be a rate-limiting step in glycolysis (143). Thus, the inhibition of glycolytic enzyme activity is in itself weak evidence for an alteration in glycolytic flux. Currently, no data exist to indicate that the diminished activity of glycolytic enzymes in peripheral nerve is reflected as a decrease in glycolytic flux. Furthermore, it is unclear whether these changes in enzyme activity precede or accompany the degeneration of axons in acrylamide-treated animals.

## *Protection Experiments*

Attempts to evaluate the hypothesis that acrylamide inhibits axonal energy transformation have been frustrated by an inability to distinguish enzyme

activities within the different cellular compartments of peripheral nerves. While it is possible selectively to remove the bulk of the connective tissue (epineurium and perineurium) surrounding nerve fibers, the remaining intrafascicular tissue contains endothelial cells, pericytes, fibroblasts, and a large number of Schwann cells intimately associated with axons. When intrafascicular tissue is homogenized, the anatomic separation between the axonal glycolytic enzyme and the non-axonal enzyme is lost. This allows any excess enzyme in non-axonal cells to obscure potential decreases in glycolytic flux that may be occurring specifically within the axonal compartment. Thus, indirect means of evaluating the hypothesis have been attempted.

One recent approach has been to investigate the effects on peripheral nerves of the repeated systemic administration of iodoacetic acid, a known inhibitor of glycolysis (M. S. Miller, P. S. Spencer, unpublished data). Measurement of glycolytic enzyme activity in the peripheral nerve demonstrated a greater degree of inhibition than that produced by doses of acrylamide high enough to precipitate nerve degeneration, yet no neuropathy resulted in the animals treated with iodoacetic acid. These data suggest that altered peripheral nerve glycolytic enzyme activity is not the sole mechanism by which acrylamide produces distal axonopathy. In addition, studies by Hashimoto and Aldridge (44) demonstrated no changes in pyruvate or lactate concentrations in brain exposed to acrylamide in vitro or in vivo.

Another indirect approach to examining the role of energy transformation in the genesis of acrylamide neuropathy has been to supplement animals with pyruvate during the period of intoxication in an attempt to circumvent the putative blockade in glycolytic function produced by acrylamide. Early studies using small numbers of animals found that administration of sodium pyruvate delayed the onset of acrylamide neuropathy (144, 145). This finding was only partially confirmed by Sterman et al (146). More recently, Dairman et al (145) have reported the results of a series of studies using a large number of animals that were subjected to detailed functional, morphological, and biochemical analyses of CNS and PNS tissues. These studies demonstrated conclusively that the administration of pyruvate, either the sodium salt or the free acid, delays the appearance of axonal degeneration and functional neuropathy. While these results are consistent with a potential role for altered glycolysis in the etiology of neuropathy, the mechanism underlying protection is unknown. The failure of exogenous pyruvate administration to prevent the development of neuropathy suggests that either the dose of pyruvate employed was submaximal or that altered glycolysis is not the sole pathologic mechanism underlying the development of this disorder.

A similar protective effect has been reported in animals supplemented with pyridoxine (vitamin $B_6$) (147). Pyridoxine is a cofactor required by many enzymatic reactions, including a number of amino-acid (aspartate, glutamate)

transaminase reactions that supply pyruvate, oxaloacetate, 2-keto-glutarate, and succinate to the tricarboxylic acid cycle, as well as enzymes regulating the metabolism of glycogen to glucose (148).

The mechanisms underlying pyruvate- and pyridoxine-induced retardation of acrylamide neuropathy are unknown. The development of acrylamide axonopathy can be delayed by several non-specific methods, including the modulation of non-protein sulfhydryl content and altered pharmacokinetics. Therefore, it is critical that the means by which exogenously administered compounds delay or prevent acrylamide axonopathy be confirmed prior to interpretation.



## SUMMARY

The neurotoxic property of acrylamide has been studied for more than 30 years. Recognition that the underlying lesion involves distal retrograde degeneration of long and large-diameter axons demonstrated that acrylamide neuropathy belongs to the class of central-peripheral distal axonopathies. This is a relatively common response of the nervous system found in a large number of unrelated toxic-metabolic states. The ready availability of pure acrylamide and the ability to reproduce a reliable model of acrylamide neuropathy in laboratory animals encouraged many investigators to focus on this disorder as a paradigm with which to study the cellular and biochemical mechanisms underlying distal axonopathies.

The discovery that abnormalities of energy-dependent axonal transport are associated with nerve fiber degeneration led investigators to explore the possibility that energy flux in axons is perturbed by the action of acrylamide. This hypothesis has proved as yet untestable by direct means, due to an inability to separate glial contributions to energy production from those of the axon. Indirect means of addressing this hypothesis have, however, yielded data suggestive of a role for altered glycolysis in the etiology of acrylamide axonopathy. However, it is becoming apparent that, if diminished glycolytic flux is involved in the etiology of axonopathy, it is unlikely to be the sole pathologic mechanism and may in fact represent only one facet of a complex series of biochemical events underlying axonopathy.

A recent hypothesis has suggested that alterations in axon-perikaryal interactions may underlie acrylamide-induced axonopathy. Evidence exists to indicate that alterations in the retrograde axonal transport of axon-derived maintenance- or repair-initiating factors may prevent or attenuate the perikaryal responses necessary to repair and maintain the distal axon in the presence of acrylamide. Thus, acrylamide-induced axonopathy may be the result of at least two biochemical lesions: (*a*) A relatively non-specific axonal lesion that may be the inhibition of glycolytic enzyme activity or some other axon lesion resulting

from the interaction of acrylamide with sulfhydryl, amino, and/or hydroxyl moieties associated with axonal constituents. (*b*) Inhibition of perikaryal-mediated axon repair and maintenance mechanisms that could occur as a result of alterations in retrograde axonal transport of one or more lesion-associated factors or as a result of a direct action of acrylamide on the perikaryon. A third hypothesis suggests that a direct action of acrylamide on the neuronal perikaryon alone may result in a generalized, non-specific metabolic lesion that stresses the neuron to a point where the distal end can no longer be maintained.

Regardless of the actual mechanism of acrylamide-induced axonopathy, recent investigations by several laboratories have provided new and exciting insights into the possible mechanism of distal axonopathy, the means by which axon-perikaryal communication occurs, and the manner in which the perikaryon responds to axon damage. In addition to the obvious significance that understanding mechanisms of toxin-induced peripheral nerve degeneration has to the toxicologist, toxic neuropathy may be a means by which the basic mechanisms underlying comparable human neurodegenerative disorders may profitably be studied.



ACKNOWLEDGEMENTS

These studies were supported by federal grants from NIH NINCDS NS19611, NIOSH OH 00851, and OH 00535.

## *Literature Cited*

1. Cavanagh, J. B. 1964. The significance of the "dying-back" process in experimental and human neurological disease. *Int. Rev. Exp. Pathol.* 3:219–67
2. Spencer, P. S., Schaumburg, H. H. 1977. Ultrastructural studies of the dying-back process. IV. Differential vulnerabilities of PNS and CNS fibers in experimental central-peripheral distal axonopathies. *J. Neuropathol. Exp. Neurol.* 36:300–20
3. Spencer, P. S., Miller, M. S., Ross, S. M., Schwab, B., Sabri, M. I. 1985. Biochemical mechanisms underlying primary degeneration of axons. In *Handbook of Neurochemistry,* ed. A. Lajtha. 9:31–66. New York: Plenum
4. Spencer, P. S., Schaumburg, H. H. 1974. A review of acrylamide neurotoxicity. II. Experimental animal neurotoxicity and pathologic mechanisms. *Can. J. Neurol. Sci.* 1:152–64
5. Le Quesne, P. M. 1980. Acrylamide. In *Experimental and Clinical Neurotoxicology,* ed. P. S. Spencer, H. H. Schaumburg, pp. 309–25. Baltimore, MD: Williams & Wilkins
6. Tilson, H. A. 1981. The neurotoxicity of acrylamide: an overview. *Neurobehav. Toxicol. Teratol.* 3:437–44
7. Spencer, P. S., Schaumburg, H. H., Sabri, M. I., Veronesi, B. 1980. The enlarging view of hexacarbon neurotoxicity. *CRC Crit. Rev. Toxicol.* 3:279–356
8. Couri, D., Milks, M. 1982. Toxicity and metabolism of the neurotoxic hexacarbons *n*-hexane, 2-hexanone, and 2,5-hexanedione. *Ann. Rev. Pharmacol. Toxicol.* 22:145–66
9. Ochs, S., Sabri, M. I., Ranish, N. 1970. Somal site of synthesis of fast transported material in mammalian nerve fibers. *J. Neurobiol.* 1:329–44
10. Singer, M., Salpeter, M. M. 1966. Transport of tritium-labelled I-histidine through the Schwann and myelin sheaths into the axon of peripheral nerves. *Nature* 210:1225–1227
11. Skene, J. H. P., Willard, M. 1981. Axonally transported proteins associated with axon growth in rabbit central and peripheral nervous systems. *J. Cell Biol.* 89:96–103
12. Skene, J. H. P., Willard, M. 1981.

Changes in axonally transported proteins during axon regeneration in toad retinal ganglion cells. *J. Cell Biol.* 89:86–95
13. Brady, S. T., Lasek, R. J. 1981. Nerve-specific enolase and creatinine phosphokinase in axonal transport: Soluble proteins and the axoplasmic matrix. *Cell* 23:515–23
14. Schwartz, J. H. 1979. Axonal transport: Components, mechanisms and specificity. *Ann. Rev. Neurosci.* 2:467–504
15. Black, M. M., Lasek, R. J. 1979. Axonal transport of actin: Slow component b is the principal source of actin for the axon. *Brain Res.* 171:401–13
16. Goldberg, D. J., Sherbany, A. A., Schwartz, J. H. 1978. Kinetic properties of normal and perturbed axonal transport of serotonin in a single identified axon. *J. Physiol.* 281:559–79
17. Ochs, S. 1975. Retention and redistribution of proteins in mammalian nerve fibers by axoplasmic transport. *J. Physiol.* 253:459–75
18. Gross, G. W., Beidler, L. M. 1975. A quantitative analysis of isotope concentration profiles and rapid transport velocities in the C-fibers of the garfish olfactory nerve. *J. Neurobiol.* 6:213–32
19. Brimijoin, S. 1975. Stop-flow: A new technique for measuring axonal transport of dopamine-B-hydroxylase. *J. Neurobiol.* 6:379–94
20. Brimijoin, S., Lundberg, J. M., Brodin, E., Hokfelt, T., Nilsson, G. 1980. Axonal transport of substance P in the vagus and sciatic nerves of the guinea pig. *Brain Res.* 191:443–57
21. Hanson, M. 1978. A new method to study fast axonal transport *in vivo*. *Brain Res.* 153:121
22. Bisby, M. A., Bulgar, V. T. 1977. Reversal of axonal transport at a nerve crush. *J. Neurochem.* 29:313–20
23. Grafstein, B. 1975. The nerve cell response to axotomy. *Exp. Neurol.* 48:32–51
24. Cragg, B. C. 1970. What is the signal for chromatolysis? *Brain Res.* 23:1–21
25. Kristensson, K., Sjostrand, J. 1972. Retrograde transport of protein tracer in rabbit hypoglossal nerve regeneration. *Brain Res.* 45:175–81
26. Pilar, G., Landmesser, L. J. 1972. Axotomy mimicked by local colchicine application. *Science* 177:1116–18
27. Singer, P. A., Mehler, S., Fernandez, H. L. 1982. Blockade of retrograde axonal transport delays the onset of metabolic and morphologic changes induced by axotomy. *J. Neurosci.* 2:1299–306
28. Droz, B., Rambourg, A., Koenig, H. L. 1975. The smooth endoplasmic reticulum: Structure and role in the renewal of axonal membranes and synaptic vesicles by fast axonal transport. *Brain Res.* 93:1–13
29. Holtzman, E., Teichberg, S., Abrahams, S. J., Citkoitz, E., Kawai, N., Peterson, E. R. 1973. Notes on synaptic vesicles and related structures, endoplasmic reticulum, lysosomes and peroxisomes in nervous tissue and adrenal medulla. *J. Histochem. Cytochem.* 21:349–85
30. Sahenk, Z., Mendell, J. R. 1978. Abnormal retrograde axoplasmic transport in the pathogenesis of experimental dying-back neuropathy of BOTZ. *Neurology* 28:357
31. Price, D. L., Griffin, J., Young, A., Peck, K., Stocks, A. 1975. Tetanus toxin: Direct evidence for retrograde intraaxonal transport. *Science* 188:945–47
32. Bunt, A. H., Haschke, R. H., Lund, R. D., Calins, D. F. 1976. Factors affecting retrograde axonal transport of horseradish peroxidase in the visual system. *Brain Res.* 102:152–55
33. Hendry, I. A., Stach, R., Herrup, K. 1974. Characteristics of the retrograde axonal transport system for nerve growth factor in the sympathetic nervous system. *Brain Res.* 82:117–28
34. Stoeckel, K., Schwab, M., Thoenen, H. 1975. Comparison between the retrograde axonal transport of nerve growth factor and tetanus toxin in motor, sensory and adrenergic neurons. *Brain Res.* 99:1–16
35. Miller, M. S., Buck, S. H., Sipes, I. G., Yamamura, H. I., Burks, T. F. 1982. Regulations of substance P by nerve growth factor: disruption by capsaicin. *Brain Res.* 250:193–96
36. Stoeckel, K., Schwab, M., Thoenen, H. 1977. Role of gangliosides in the uptake and retrograde axonal transport of cholera and tetanus toxin as compared to nerve growth factor and wheat germ agglutinin. *Brain Res.* 132:273–85
37. Fink, D. J., Gainer, H. 1980. Retrograde axonal transport of endogenous proteins in sciatic nerve demonstrated by covalent labelling in vivo. *Science* 208:303
38. Porcellati, G. 1969. Peripheral nerve. See Ref. 3, 11:393–422
39. Bikales, N. M., Kolodny, E. R. 1963. Acrylamide. In *Encyclopedia of Chemical Technology*, ed. A. Standen, pp. 274–84. New York: Interscience. 2nd ed.
40. Bikales, N. M. 1970. Acrylamide and related amides. In *High Polymers, Vinyl and Diene Monomers*, ed. E. C. Leonard, pp. 81–104. New York: Wiley

41. Cavins, J. F., Friedman, M. 1967. Specific modification of protein sulfhydryl groups with alpha,beta-unsaturated compounds. *Fed. Proc.* 26:822
42. Cavins, J. F., Friedman, M. 1967. New amino acids derived from reaction of E-amino groups in protein with alpha,beta-unsaturated compounds. *Biochemistry* 6: 3766–70
43. Druckery, H., Consbruch, U., Schmahl, D. 1953. Effects of monomeric acrylamide on proteins. *Z. Naturforsch.* 86: 145–50
44. Hashimoto, K., Aldridge, W. N. 1970. Biochemical studies on acrylamide, a neurotoxic agent. *Biochem. Pharmacol.* 19:2591–604
45. Auld, R. B., Bedwell, S. F. 1967. Peripheral neuropathy with sympathetic overactivity from industrial contact with acrylamide. *Can. Med. Assoc. J.* 96: 652
46. Garland, T. O., Patterson, M. H. 1967. Six cases of acrylamide poisoning. *Br. Med. J.* 4:134–38
47. Sumner, A. J., Asbury, A. K. 1975. Physiological studies of the dying-back phenomenon. Muscle stretch afferents in acrylamide neuropathy. *Brain* 98:91–100
48. Schaumburg, H. H., Wisniewski, H. M., Spencer, P. S. 1974. Ultrastructural studies of the dying-back process: I. Peripheral nerve terminal and axon degeneration in systemic acrylamide intoxication. *J. Neuropathol. Exp. Neurol.* 33:260–84
49. Kesson, C. M., Baird, A. W., Lawson, D. H. 1977. Acrylamide poisoning. *Postgrad. Med. J.* 53:16–17
50. Fullerton, P. M. 1969. Electrophysiological and histological observations on peripheral nerves in acrylamide poisoning in man. *J. Neurol. Neurosurg. Psych.* 32:186–92
51. Kuperman, A. S. 1958. Effects of acrylamide on the central nervous system of the cat. *J. Pharmacol. Exp. Ther.* 123:180–92
52. Leswing, R. J., Ribelin, W. E. 1969. Physiologic and pathologic changes in acrylamide neuropathy. *Arch. Environ. Health* 18:22–29
53. McCollister, D. D., Oyen, F., Rowe, V. K. 1964. Toxicology of acrylamide. *Toxicol. Appl. Pharmacol.* 6:172–81
54. Fullerton, P. M., Barnes, J. M. 1966. Peripheral neuropathy in rats produced by acrylamide. *Br. J. Indust. Med.* 23:210–21
55. Suzuki, K., Pfaff, L. D. 1973. Acrylamide neuropathy in rats—An electron microscopic study of degeneration and regeneration. *Acta Neuropath.* 24:197–213
56. Tilson, H. A., Cabe, P. A., Spencer, P. S. 1979. Acrylamide behavioral toxicity in rats: A correlated neurobehavioral and pathological study. *Neurotoxicology* 1: 89–104
57. Bradley, W. G., Asbury, A. K. 1970. Radioautographic studies of Schwann cell behavior. I. Acrylamide neuropathy in the mouse. *J. Neuropathol. Exp. Neurol.* 29:500–6
58. Evans, H., Teal, J. J. 1981. Appetitive behaviors as models of the neurotoxicity of acrylamide. *Fed. Proc.* 40:677
59. Gilbert, S. G., Maurissen, J. P. 1982. Assessment of the effects of acrylamidc, methylmercury, and 2,5-hexanedione on motor functions in mice. *J. Toxicol. Environ. Health* 10:31–41
60. Hopkins, A. P. 1970. The effect of acrylamide on the peripheral nervous system of the baboon. *J. Neurosurg. Psychiatr.* 33:805–16
61. Edwards, P. M. 1975. Neurotoxicity of acrylamide and its analogues and effects of these analogues and other agents on acrylamide neuropathy. *Br. J. Ind. Med.* 32:31–38
62. Edwards, P. M. 1975. The distribution and metabolism of acrylamide and its neurotoxic analogues in rats. *Biochem. Pharmacol.* 24:1277–82
63. Miller, M. J., Carter, D. E., Sipes, I. G. 1982. The pharmacokinetics of acrylamide in Fisher 344 rats. *Toxicol. Appl. Pharmacol.* 63:36–44
64. Pastoor, T., Richardson, R. J. 1981. Blood dynamics of acrylamide in rats. *Toxicologist* 1:53
65. Rylandcr-Yueh, L. A., Cartcr, D. E. 1982. Tissuc disposition of acrylamide in multiply-dosed 5-week vs. 11-week male Holtzman rats. *Toxicologist* 2:1–50
66. Pastoor, T., Heydens, W., Richardson, R. J. 1980. Time and dose-related excretion of acrylamide metabolites in the urine of Fisher 344 rats. In *Mechanisms of Toxicity and Hazard Evaluation Developments in Toxicology and Environmental Science, Vol. 8,* ed. B. Holmstedt, R. Lauwerys, M. Mercier, M. Roberfraid. New York: Elsvier-North Holland
67. Dixit, R., Seth, P. K., Mukhtar, H. 1980. Brain glutathione-S-transferase catalyzed conjugation of acrylamide: A novel mechanism for detoxification of neurotoxin. *Biochem. Int.* 1:547–52
68. Dixit, R., Husain, R., Mukhtar, H., Seth, P. K. 1981. Acrylamide induced inhibition of hepatic glutathione-S-

transferase activity in rats. *Toxicol. Letts.* 7:207–10

69. Dixit, R., Husain, R., Seth, P. K., Mukhtar, H. 1980. Effect of diethyl maleate on acrylamide induced neuropathy in rats. *Toxicol. Letts.* 6:417–21
70. Dixit, R., Mukhtar, H., Seth, P. K., Krishna, C. R. 1980. Binding of acrylamide with glutathione-S-transferases. *Chem. Biol. Interact.* 32:353–59
71. Mukhtar, H., Dixit, R., Seth, P. K. 1981. Reduction in cutaneous and hepatic glutathione contents, glutathione-S-transferase and aryl hydrocarbon hydroylase activities following topical application of acrylamide to mouse. *Toxicol. Letts.* 9:153–56
72. Das, M., Mukhtar, H., Seth, P. K. 1982. Effect of acrylamide on brain and hepatic mixed-function oxidase and glutathione-S-transferase in rats. *Toxicol. Appl. Pharmacol.* 66:420–26
73. Kaplan, M. L., Murphy, S. D., Gilles, F. H. 1973. Modification of acrylamide neuropathy in rats by selected factors. *Toxicol. Appl. Pharmacol.* 24:564–79
74. Hashimoto, K., Sakamoto, J., Tannii, H. 1981. Neurotoxicity of acrylamide and related compounds and their effects on male gonads in mice. *Arch. Toxicol.* 47:179–89
75. Howland, R. D. 1981. The etiology of acrylamide neuropathy: Enolase, phosphofructokinase, and glyceraldehyde-3-phosphate dehydrogenase activity in peripheral nerve, spinal cord, brain and skeletal muscle of acrylamide-intoxicated cats. *Toxicol. Appl. Pharmacol.* 60:324–33
76. Howland, R. D., Vyas, I. L., Lowndes, H. E. 1980. The etiology of acrylamide neuropathy: Possible involvement of neuron specific enolase. *Brain Res.* 190:529–35
77. Howland, R. D., Vyas, I. L., Lowndes, H. E., Argentieri, T. M. 1980. The etiology of toxic peripheral neuropathies: In vitro effects of acrylamide and 2,5-hexanedione on brain enolase and other glycolytic enzymes. *Brain Res.* 202:131–42
78. Kemplay, S., Cavanagh, J. B. 1984. Effects of acrylamide and other sulfhydryl compounds in vivo and in vitro on staining of motor nerve terminals by the zinc iodide-osmium technique. *Muscle Nerve* 7:94–100
79. Kemplay, S., Cavanagh, J. B. 1984. Effects of acrylamide and some other sulfhydryl reagents on spontaneous and pathologically induced terminal sprouting from motor end-plates. *Muscle Nerve* 7:101–9
80. Sabri, M. I., Spencer, P. S. 1980. Inhibition of glyceraldehyde-3-phosphate and other glycolytic enzymes by acrylamide. *Neurosci. Letts. Suppl.* 5:455
81. Sabri, M. I., Moore, C. L., Spencer, P. S. 1979. Studies on the biochemical basis of distal axonopathies I. Inhibition of glycolysis by neurotoxic hexacarbon compounds. *J. Neurochem.* 32:683–89
82. Sabri, M. I. 1983. Mechanism of action of acrylamide on the nervous system. *Biol. Mem.* 8:16–27
83. Prineas, J. 1969. The pathogenesis of dying-back polyneuropathies. II. An ultrastructural study of experimental acrylamide intoxication in the cat. *J. Neuropathol. Exp. Neurol.* 28:598–621
84. Spencer, P. S., Thomas, P. K. 1974. Ultrastructural studies of the dying-back process. II. The sequestration and removal by Schwann cells and oligodendrocytes of organelles from normal and diseased axons. *J. Neurocytol.* 3:763–83
85. Hanna, R. B., Spencer, P. S., Pappas, G. D. 1976. Structural differences in membranes in the pacinian corpuscle. A freeze-fracture study. *Neurosci. Abstr.* 2:404
86. Pleasure, D. E., Mishner, K. C., Engel, W. K. 1969. Axonal transport of proteins in experimental neuropathies. *Science* 166:524–25
87. Bradley, W. G., Williams, M. H. 1973. Axoplasmic flow in axonal neuropathies. *Brain* 96:235–46
88. Weir, R. L., Glaubiger, G., Chase, T. N. 1978. Inhibition of fast axoplasmic transport by acrylamide. *Environ. Res.* 17:251–55
89. Souyri, F., Chretien, M., Droz, B. 1981. Retention of proteins conveyed with fast axonal transport in acrylamide-induced neuropathy. I. Light radioautographic study: Focal retention of fast transported proteins in preterminal axons. *Brain Res.* 205:1–13
90. Chretien, M., Patey, G., Souyri, F., Droz, B. 1981. Acrylamide induced neuropathy and impairment of axonal transport of proteins. II. Abnormal accumulations of smooth endoplasmic reticulum at sites of focal retention of fast transported proteins. Electron microscope radioautographic study. *Brain Res.* 205:15–28
91. Sidenius, P., Jakobsen, J. 1983. Anterograde axonal transport in rats during intoxication with acrylamide. *J. Neurochem.* 40:697–704

92. Sumner, A., Pleasure, D., Ciesielka, K. 1976. Slowing of fast axoplasmic transport in acrylamide neuropathy. *J. Neuropathol. Exp. Neurol.* 35:319 (Abstr.)
93. Griffin, J. W., Price, D. L. 1976. Axonal transport in motor neuron pathology. In *Recent Research Trends*, ed. J. Andrews, R. Johnson, M. Brazier, p. 33–67. New York: Academic
94. Couraud, J. Y., Di Giamberardino, J., Chretien, M., Souyri, F., Fardeau, M. 1982. Acrylamide neuropathy and changes in the axonal transport and muscular content of the molecular forms of acetylcholinesterase. *Muscle Nerve* 5:302–12
95. Schotman, P., Gipon, L., Jennekens, F. G. I., Gispen, W. H. 1978. Polyneuropathies and CNS protein metabolism III. Changes in protein synthesis rate induced by acrylamide intoxication. *J. Neuropathol. Exp. Neurol.* 37:820–37
96. Hashimoto, K., Ando, K. 1973. Alteration of amino acid incorporation into proteins of the nervous system in vitro after administration of acrylamide to rats. *Biochem. Pharmacol.* 22:1057–66
97. Sahenk, Z., Mendell, J. R. 1981. Acrylamide and hexanedione neuropathies: Abnormal bidirectional transport rate in distal axons. *Brain Res.* 219:397–405
98. Jakobsen, J., Sidenius, P. 1983. Early and dose-dependent decrease of retrograde axonal transport in acrylamide-intoxicated rats. *J. Neurochem.* 40:447–54
99. Miller, M. S., Miller, M. J., Burks, T. F., Sipes, I. G. 1983. Altered retrograde axonal transport of nerve growth factor after single and repeated doses of acrylamide in the rat. *Toxicol. Appl. Pharmacol.* 69:96–101
100. Miller, M. S., Spencer, P. S. 1984. Single doses of acrylamide reduce retrograde transport velocity. *J. Neurochem.* 43(5): 1401–8
101. Kemplay, S., Cavanagh, J. B. 1983. Effects of acrylamide and botulinum toxin on horseradish peroxidase labelling of trigeminal motor neurons in the rat. *J. Anat.* 137:477–82
102. Gold, B. G., Griffin, J. W., Price, D. L. 1983. Slow axonal transport following single high-dose acrylamide administration. *Soc. Neurosci. Abstr.* 9:669
103. Kreutzberg, G. W. 1968. Histochemical demonstration of a colchicine-induced blockage of enzyme transport in axons of peripheral nerves. *Proc. 3rd Int. Congr. Histochem. Cytochem.*, pp. 133–34. New York: Springer-Verlag
104. Kreutzberg, G. W. 1969. Neuronal dynamics and axonal flow. IV. Blockage of intra-axonal enzyme transport by colchicine. *Proc. Natl. Acad. Sci. USA* 62: 722–28
105. Karlsson, J. O., Hanson, H. A., Sjostrand, J. 1971. Effect of colchicine on axonal transport and morphology of retinal ganglion cells. *Z. Zellforsch. Mikrosk. Anat.* 115:265–83
106. Karlsson, J. O., Sjostrand, J. 1969. The effect of colchicine on the axonal transport of protein in the optic nerve and tract of the rabbit. *Brain Res.* 13:617–19
107. Hanson, M., Edstrom, A. 1978. Mitosis inhibitors and axonal transport. *Int. Rev. Cytol.* 7:373–402
108. Samson, F. E. 1976. Pharmacology of drugs that affect intracellular movement. *Ann. Rev. Pharmacol. Toxicol.* 16:143–59
109. Morgan-Hughes, J. A., Sinclair, S., Durston, J. H. J. 1974. The pattern of peripheral nerve regeneration induced by crush in rats with severe acrylamide neuropathy. *Brain* 97:235–50
110. Griffin, J. W., Price, D. L., Drachman, D. B. 1977. Impaired axonal regeneration in acrylamide intoxication. *J. Neurobiol.* 8:355–70
111. Cavanagh, J. B., Gysbers, M. F. 1980. "Dying-back" above a nerve ligature produced by acrylamide. *Acta Neuropathol.* 51:169–77
112. Benowitz, L. I., Sashoua, V. E., Yoon, M. G. 1981. Rapidly transported proteins in the regenerating optic nerve of goldfish. *J. Neurosci.* 1:300–7
113. Bisby, M. A. 1980. Changes in the composition of labeled protein transported in motor axons during their regeneration. *J. Neurobiol.* 11:435–45
114. Giulian, D., Des Ruisseaux, H., Cowburn, D. 1980. Biosynthesis and intra-axonal transport of proteins during neuronal regeneration. *J. Biol. Chem.* 255:6494–501
115. Perry, G. W., Wilson, D. L. 1981. Protein synthesis and axonal transport during nerve regeneration. *J. Neurochem.* 37: 1203–17
116. Perry, G. W., Krayanek, S. R., Wilson, D. L. 1983. Protein synthesis and rapid axonal transport during regrowth of dorsal root axons. *J. Neurochem.* 40:1590–98
117. Gunning, P. W., Kaye, P. L., Austin, L. 1977. In vivo RNA synthesis within the rat nodose ganglia. *J. Neurochem.* 28: 1237–40
118. Gunning, P. W., Kaye, P. L., Austin, L. 1977. In vivo synthesis of rapidly labelled RNA within the rat nodose gan-

glia following vagotomy. *J. Neurochem.* 28:1245–48

119. Kaye, P. L., Gunning, P. W., Austin, L. 1977. In vivo synthesis of stable RNA within the rat nodose ganglia following vagotomy. *J. Neurochem.* 28:1241–43
120. Langford, C. J., Scheffer, J. W., Jeffrey, P. L., Austin, L. 1980. The in vitro synthesis of RNA within the rat nodose ganglion following vagotomy. *J. Neurochem.* 34:531–39
121. Miller, M. S., Spencer, P. S. Inhibition by acrylamide of increased ornithine decarboxylase activity and RNA synthesis in dorsal root ganglion following sciatic nerve transsection. *J. Neurochem.* Submitted for publication
122. Russell, D. H., Snyder, S. H. 1968. Amine synthesis in rapidly growing tissues: Ornithine decarboxylase in rat liver. *Endocrinology* 86:1414–19
123. Russell, D. H. 1980. Ornithine decarboxylase as a biological and pharmacological tool. *Pharmacology* 20:117–29
124. Slotkin, T. A. 1979. Ornithine decarboxylase as a tool in developmental neurobiology. *Life Sci.* 24:1623–30
125. Slotkin, T. A., Seidler, F. J., Whitmore, W. L., Weigel, S. J., Slepetis, R. J., et al. 1983. Critical eriods for the role of ornithine decarboxylase and the polyamines in growth and development of the rat: Effects of exposure to alpha-difluoromethylornithine during discrete prenatal or postnatal intervals. *Int. J. Devl. Neurosci.* 1:113–127
126. Slotkin, T. A., Whitmore, W. L., Lerea, L., Slepetis, R. J., Weigel, S. J., et al. 1983. Role of ornithine decarboxylase and the polyamines in nervous system development: Short-term postnatal administration of alpha-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase. *Int. J. Dev. Neurosci.* 1:7–16
127. Ingoglia, N. A., Sturman, J. A., Eisner, R. A. 1977. Axonal transport of putrescine, spermine, and spermidine in normal and regenerating goldfish optic nerves. *Brain Res.* 130:433–45
128. Rochel, S., Margolis, F. L. 1980. The response of ornithine decarboxylase during neuronal degeneration and regeneration in olfactory epithelium. *J. Neurochem.* 35:850–60
129. Russell, D. H. 1973. Polyamines in growth—Normal and neoplastic. In *Polyamines in Normal and Neoplastic Growth,* ed. D. H. Russell, pp. 1–13. New York: Raven
130. Kohsaka, S., Heacock, A. M., Klinger, P. D., Porta, R., Agranoff, B. W. 1982. Dissociation of enhanced ornithine decarboxylase activity and optic nerve regeneration in goldfish. *Dev. Brain Res.* 4:149–56
131. Kohsaka, S., Schwartz, M., Agranoff, B. W. 1981. Increased activity of ornithine decarboxylase in goldfish following optic nerve crush. *Dev. Brain Res.* 1:391–401
132. Sterman, A. B. 1982. Cell body remodeling during dying-back axonopathy: DRG changes during advanced disease. *J. Neuropathol. Exp. Neurol.* 41:400–11
133. Sterman, A. B. 1982. Acrylamide induces early morphologic reorganization of the neuronal cell body. *Neurology* 32:1023–26
134. Sterman, A. B. 1983. Altered sensory ganglia in acrylamide neuropathy: Quantitative evidence of neuronal reorganization. *J. Neuropathol. Exp. Neurol.* 42:166–76
135. Cavanagh, J. B. 1982. The pathokinetics of acrylamide intoxication: A reassessment of the problem. *Neuropathol. Appl. Neurobiol.* 8:315–36
136. Cavanagh, J. B., Gysbers, M. F. 1983. Ultrastructural features of the purkinje cell damage caused by acrylamide in the rat: a new phenomenon in cellular neuropathology. *J. Neurocytol.* 12:413–37
137. Ochs, S. 1982. In *Axoplasmic Transport and its Relation to Other Nerve Functions,* pp. 148–77. New York: Wiley
138. Griffin, J. W., Hoffman, P. N., Price, D. L. 1982. Axoplasmic transport in B,B'-Iminodipropionitrile neuropathy. In *Axoplasmic Transport in Physiology and Pathology,* ed. D. G. Weiss, A. Gorio, pp. 109–18. Berlin: Springer-Verlag
139. Tanii, H., Hashimoto, K. 1983. Neurotoxicity of acrylamide and related compounds in rats: Effects on rotorod performance, morphology of nerves and neurotubulin. *Arch. Toxicol.* 54:203–13
140. Sabri, M. I., Ochs, S. 1971. Inhibition of glyceraldehyde-3-phosphate dehydrogenase in mammalian nerve by iodoacetate. *J. Neurochem.* 13:1509–14
141. Spencer, P. S., Sabri, M. I., Schaumburg, H. H., Moore, C. L. 1978. Does a defect of energy metabolism in the nerve fiber underlie axonal degeneration in polyneuropathies? *Ann. Neurol.* 5:501–7
142. Spencer, P. S., Sabri, M. I., Politis, M. 1980. Methyl-*n*-butyl ketone, carbon disulfide, and acrylamide: Putative mechanism of neurotoxic damage. In *Advances in Neurotoxicology,* ed. L. Manzo, pp. 173–80. New York: Pergamon
143. Lehninger, A. L. 1975. *Biochemistry.* New York: Worth. 2nd ed.

144. Sabri, M. I., Dairman, W., Juhasz, L., Bischoff, M. C., Spencer, P. S. 1981. Is acrylamide neurotoxicity pyruvate sensitive? *Trans. Am. Soc. Neurochem.* 12:155
145. Dairman, W., Sabri, M. I., Bischoff, M., Juhasz, L., Ng, T., Spencer, P. S. 1984. Dietary pyruvate suppresses acrylamide neuropathy in male rats. *Ann. Neurol.* Submitted for publication
146. Sterman, A. B., Panasci, D. J., Persons, W. 1983. Does pyruvate prevent acrylamide neurotoxicity? Implications for disease pathogenesis. *Exp. Neurol.* 82:148–58
147. Loeb, A. L., Anderson, R. J. 1981. Antagonism of acrylamide neurotoxicity by supplementation with vitamin $B_6$. *Neurotoxicology* 2:625–33
148. Metzler, D. E. 1977. *Biochemistry. The Chemical Reactions of Living Cells*. New York: Academic