RADIOAUTOGRAPHIC STUDIES OF SCHWANN CELL 
BEHAVIOR: I. ACRYLAMIDE NEUROPATHY 
IN THE MOUSE* †

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Acrylamide (CH\_2 = CH·CO·NH\_2) is a water soluble compound, the polymer of which has found several industrial uses. Early experimental studies revealed neurotoxicity which was thought to be central in type (10, 12), although the peripheral nerves were not then examined. Fullerton and Barnes (7), in 1966, demonstrated severe distal axonal degeneration in acrylamide treated rats. Similar changes have been found in baboons (9) and cats (11). A few human cases, usually resulting from industrial exposure, have been described (4, 6, 8).

This particular experimental neuropathy was selected for radioautographic study in order to determine the timing and extent of Schwann cell proliferation, especially in comparison to the same events in Wallerian degeneration. In addition, one might with these techniques attempt to decipher the relation of Schwann cell proliferation to other events in nerve fiber breakdown. Since acrylamide neuropathy is an almost pure axonal degeneration, it is hoped that knowledge of cellular events in its course might be more generally applied to human axonal degenerations such as those associated with uremia, vitamin deficiency, nitrofurantoin intoxication, and others.

MATERIALS AND METHODS

Young adult female mice (Balb c Gue/J × SJL/J and SJL/J strains) were used. Acrylamide was added to the drinking water in a concentration of 250 p.p.m. Animals were allowed to drink this acrylamide solution ad libitum for the first 45 days of the experiment, and thereafter received only fresh water. Mice were examined regularly for neurological abnormality. Pairs of animals were killed at random at 23, 30, 35, 40, 45, 50, 55, 60, 90, 120, and 165 days. Additional pairs of animals were studied at 30 and 45 days, and a pair of normal mice from the same shipments were studied as controls. Mice were injected intraperitoneally with tritiated thymidine (5.0μc/gm. body weight; specific activity 3.0 c/mM Schwarz Bio-Research) 1 hour prior to sacrifice by decapitation. Sciatic nerves from the level of the sciatic notch to the tibial head were quickly dissected, slightly stretched on paper cards, and fixed for 24 hours at 4°C in 3.6 per cent glutaraldehyde in 0.1M phosphate buffer at pH 7.5. Radioautographs of longitudinal sections of one sciatic nerve were prepared. Two blocks were cut from the distal part of the nerve after discarding the terminal 2mm, de-
hydrated in 3 changes of methanol:methylcellosolve (1:1, V/V), and embedded in glycol methacrylate (2). Two micron sections were cut with a glass knife using a Porter-Blum MT-1 microtome, floated in a drop of water on glass slides and allowed to dry in air. All radioautographs were dipped in total darkness in Kodak NTB-2 liquid photographic emulsion, exposed for 3 weeks at −70°C in light-tight boxes and developed in a single run. The preparations were developed in Kodak D19 developer for 3 minutes, and stained with 0.05 per cent toluidine blue. Similar blocks from the contralateral sciatic nerve were post-fixed for 4 hours in 2 per cent osmic acid solution in 0.1M phosphate buffer at pH 7.5, dehydrated with graded ethanol mixtures, and embedded in epon. Longitudinal sections of 2μ thickness were stained with 1 per cent para-phenylenediamine for 5 to 8 minutes, dried in air, and mounted for microscopy.

The labeling index of Schwann cell nuclei was determined by counting 500 to 2000 Schwann cell nuclei per animal using a ×100 oil immersion objective. An arbitrary threshold of 4 silver grains per nucleus was used; background counts were low (fig. 1). Criteria for the identification of Schwann cells and the problem of endoneurial fibroblasts are detailed in previous publications (2, 3).

An estimate of the histological severity of the neuropathy in longitudinal sections of the contralateral sciatic nerve of each animal was made using the arbitrary categories of "mild", "moderate" and "severe" (fig. 2).

The brain, spinal cord, and hamstring and gastrocnemius muscles of animals studied from the 30th to 55th day of the experiment were dissected after fixation in 10 per cent formalin in saline for 7 days. Multiple paraffin sections of the brain and cord stained with hema-

![Fig. 1. Radioautograph showing 2 labeled Schwann cell nuclei in mouse sciatic nerve with early nerve fiber breakdown 35 days after beginning acrylamide. Background silver grain counts are low. Toluidine blue stain; 3 weeks exposure; 650 ×.](http://jnen.oxfordjournals.org/)
toxylin and eosin, cresyl violet, Loyez myelin, and Bodian axon stains, and of muscle stained with hematoxylin and eosin and phosphotungstic acid-hematoxylin were examined.

RESULTS

Clinical: No animal died during intoxication with acrylamide at this dosage. The first abnormality appeared after 20 days of acrylamide administration, when about two-thirds of the animals exhibited scissoring of the hind-limbs when hanging by the tail, and had difficulty in grasping and walking along the edge of the cage because of difficulty in using their hind-limbs. After 25 days all animals appeared unaware of the position of their hind-limbs which often flexed up onto the dorsal region or they dragged their feet behind when walking. Other animals had a high-stepping gait. After 35 days, although the mice were still active, they appeared jittery, had a hoarse squeak, and had lost some weight and hair. The degree of hind-limb abnormality did not increase after 25 days. Fore-limb weakness was equivocal. Within 5 days of the withdrawal of acrylamide, gait had improved and some weight was regained. A degree of abnormality of positioning of the lower limbs persisted for a further 15 days.

General Histopathologic Observations: An occasional degenerating myelinated fiber was present in animals sacrificed 23 days after commencement of acrylamide. The proportion of degenerating fibers rose with increased duration of acrylamide intoxication, although there was considerable variation between animals. Representative areas of a normal sciatic nerve, and ones classified as having “mild”, “moderate” and “severe” neuropathy are illustrated in Figure 2.

Despite a severe degree of myelinated fiber breakdown amounting to more than half of the fibers in some animals, it proved impossible to find convincing abnormality in multiple sections of lumbar spinal cord. A certain number of microgliaicytes and densely staining shrunken motor neurons were seen in the anterior horns, but a similar number were seen in control material. The hamstring and gastrocnemius muscles were macroscopically wasted, although microscopically the changes were restricted to a slightly increased number of areas of segmental necrosis and regeneration compared to control material. Grouped atrophy of denervation was not seen in material studied up to the 55th day of the experiment.

Radioautographic Observations: The rise and fall of labeling index in Schwann cells of distal sciatic nerve is indicated in Figure 3. Labeling had begun to rise by 23 days, at which time a few myelinated fibers were degenerating. A maximum labeling index of 3.4 per cent was observed at 30 days. After 45 days Schwann cell proliferation appeared to slow down, coincident with acrylamide withdrawal. In normal adult mouse sciatic nerve only 1 labeled Schwann nucleus was found among 1200 unlabeled nuclei. The 2 zero-time specimens, although represented as histologically “mild” in grade in Figure 3, were in fact entirely normal (fig. 2a). It was not possible from this material to decide whether light-microscopic evidence of myelin breakdown preceded
Fig. 2. Longitudinal 2 micron sections of osmium-fixed, open-embedded mouse sciatic nerve illustrating histological grading of nerve fiber breakdown in acrylamide neuropathy: A. normal; B. mild; C. moderate; and D. severe. Grading is based primarily on the proportion of fibers involved, and was carried out by each investigator independently with good agreement. 1 per cent paraphenylene-diamine stain; 800 X.

proliferative activity in Schwann nuclei, but both events appeared to begin closely together in time. The Schwann labeling index appeared to relate more to the duration of exposure to acrylamide than to the degree of breakdown of myelinated fibers (fig. 3). In some animals killed at early times, nerve fiber
breakdown was extensive although Schwann cell proliferation was only moderate, while in other animals at the same time points, Schwann cell proliferation appeared to exceed nerve fiber damage. The possibility exists that in the former instance Schwann cell proliferation had already passed its peak. The continued presence of myelin debris long after withdrawal of acrylamide did not excite continued Schwann cell proliferative activity.

DISCUSSION

This radioautographic study of an experimental acrylamide neuropathy in mice has shown that almost concurrently with the appearance of clinical neuropathic signs, proliferative changes are taking place in the Schwann cells of sciatic nerve. Myelin breakdown accompanied proliferation, but it was not possible to assign primacy to either event. A maximum labeling index was reached in 10 days after the appearance of clinical signs and subsided almost as rapidly. Whether this high level of Schwann cell proliferation would have persisted if acrylamide administration had been continued is uncertain.

Although Schwann cell proliferation subsided rapidly, myelin breakdown products continued to be evident at time points subsequent to the cessation of acrylamide administration. The work of Abercrombie and Johnson (1) suggests that the stimulus to Schwann cell proliferation in Wallerian degeneration is most likely the presence of myelin or axonal breakdown products. It is
of interest that in our study, Schwann cell proliferation was already on the wane at a time when myelin and axonal breakdown persisted. Perhaps the initiation of nerve fiber and myelin destruction is the stimulus to Schwann cell proliferation rather than the persistence of myelin and axonal debris.

One is tempted to compare the cellular events in acrylamide neuropathy to those occurring in Wallerian degeneration. In the latter, the proliferative phase in Schwann cell nuclei begins in the mouse by 19 hours after nerve section and reaches a zenith of 13.5 per cent labeling index by the third day (5). In contrast, in acrylamide neuropathy, proliferation of Schwann cells occurs later, is less intense, and extends over a longer period of time. Even if one takes the appearance of clinical signs at 20 days in acrylamide-treated mice as the onset of neuropathy, the march of events is still slower than in Wallerian degeneration. This difference probably reflects the synchrony of axonal damage in Wallerian degeneration on the one hand, and the asynchrony and temporal spread of axonal damage in acrylamide neuropathy on the other.

The mechanism by which acrylamide causes nerve fiber degeneration is as yet uncertain. There is a growing belief that distal nerve fiber breakdown in the various axonal degenerations (i.e., neuropathies, associated with triorthocresyl phosphate intoxication, nitrofurantoin, isoniazid, uremia, vitamin deficiency and others) may be the result of primary metabolic events taking place in the nerve cell body. Our study and others (7, 12) have shown no abnormality of the anterior horn cells and dorsal root ganglion cells by light microscopy. Not even central chromatolysis, a phenomenon one might expect to see, was observed despite breakdown of a majority of sciatic nerve fibers distally. Critical electron microscopic studies of this experimental neuropathy have not yet been reported.*

In a recent study of axoplasmic flow in experimental toxic neuropathies, Pleasure et al. (13) have obtained evidence of impaired axonal transport in acrylamide neuropathy in cats. If the primary event in this neuropathy is a failure to produce or to transport vital substance from the perikaryon, as the work of Pleasure et al. suggests, then the concept that axonal degenerations result from some disordered metabolic event proximally is strengthened.

SUMMARY

Schwann cell proliferation in experimental acrylamide neuropathy in mice was studied by radioautography using tritiated thymidine. The animals received drinking water containing 250 p.p.m. of acrylamide for 45 days. The Schwann cell labeling index rose by 23 days and reached a peak of 3.4 per cent at 30 days. After 45 days, the Schwann cell labeling index fell, despite the continued presence of myelin breakdown products. The labeling index appeared more dependent upon the duration of exposure to acrylamide than to the extent of nerve fiber breakdown.

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REFERENCES


ANNOUNCEMENTS

The Annual "Weil Award" of $250.00 for the best paper presented at the 46th Meeting of the American Association of Neuropathologists was given to Drs. K. and Y. Suzuki, who reported on "Krabbe's Globoid Cell Leucodystrophy: Deficiency of Galactocerebroside β-Galactosidase Activity."

At the 46th Annual Meeting of the American Association of Neuropathologists, held in Atlantic City, N.J., on June 12–14, 1970, the following Officers were elected for 1970–71:

President: Lucien Rubinstein, M.D.
President-Elect: Stanley M. Aronson, M.D.
Vice-President: Leopold Liss, M.D.
Secretary-Treasurer: Edward P. Richardson, Jr., M.D.
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