percent glutaraldehyde in Sorenson's buffer, *p*H 7.3, for 2 hours, followed by washing in Sorenson's buffer overnight, followed by 90 minutes post fixation in 1 percent OsO_4 in Sorenson's buffer. Tissue was dehydrated in graded ethanol solutions and embedded in Epon 812. Thin sections were stained with lead citrate for 2 to 4 minutes [E. Reynolds, *J. Cell Biol.* **17**, 208 (1963)] followed by 20 percent uranyl acetate in methyl alcohol for 10 to 15 minutes. Tissue was examined with a Phillips 200 electron microscope operating at 60 kv.

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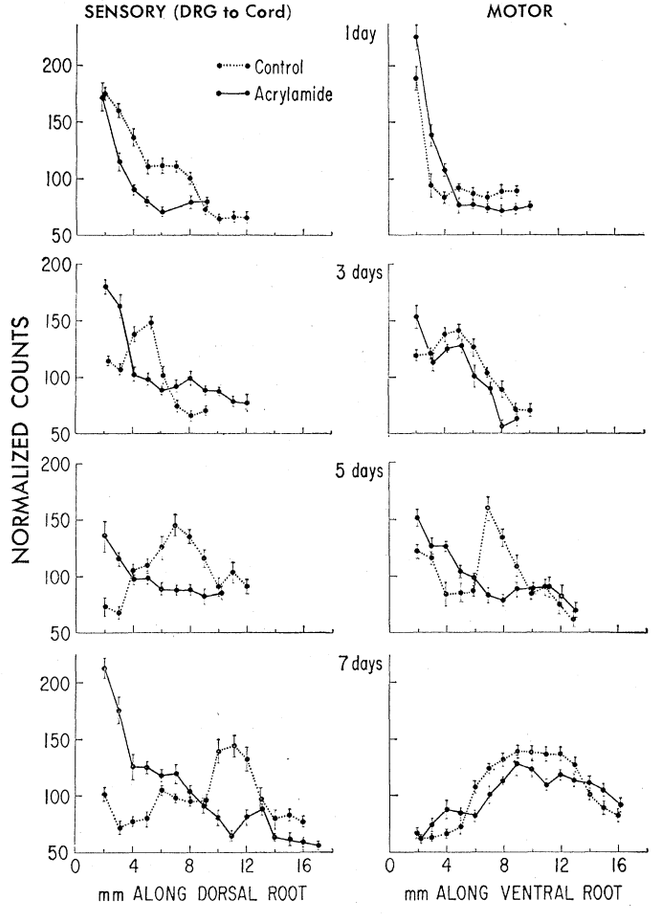
Axonal Transport of Proteins in Experimental Neuropathies

Abstract. *Axoplasmic flow of proteins is interrupted in cats with neuropathy induced by acrylamide, but it is not interrupted in normal cats and in those with neuropathy induced by tri-orthocresyl phosphate. The proteins move from lumbosacral motor neurons along the ventral roots and from ganglion cells toward the spinal cord along the dorsal roots at about 1½ millimeters per day.*

Widespread axonal destruction resembling Wallerian degeneration without the death of nerve cell bodies is seen in the neuropathies associated with thiamine deficiency, isoniazid administration, and exposure to acrylamide, tri-orthocresyl phosphate (TOCP), and other toxins (*1*). The pathogenesis of this selective axonal lesion is unknown. Transport of intracellular proteins from site of synthesis to site of function is accentuated in neurons by the extreme length of their axonal processes (*2*). Interruption of transport could result in breakdown of the axon. To test this hypothesis, flow rates of newly synthesized proteins within sensory and motor axons of normal cats were compared with those in cats with toxic neuropathies.

Neuropathies were induced by administration of acrylamide (20 mg per kilogram of body weight; orally 5 days per week) or TOCP (0.25 ml per kilogram of body weight; intramuscularly every 2 weeks). Foot drop and an unsteady, wide-based gait appeared 2 or more weeks after the beginning of treatment with either agent, but perception of a pinprick was not impaired. Axonal degeneration, predominantly distal, was found in hind limb nerves of both groups after neurologic signs had appeared. No alterations in the cell bodies of motor or sensory neurons were evident in sections stained for Nissl substance.

Flow rates of axonal protein in motor and sensory nerves were determined in 9 cats with acrylamide neuropathy, in 6



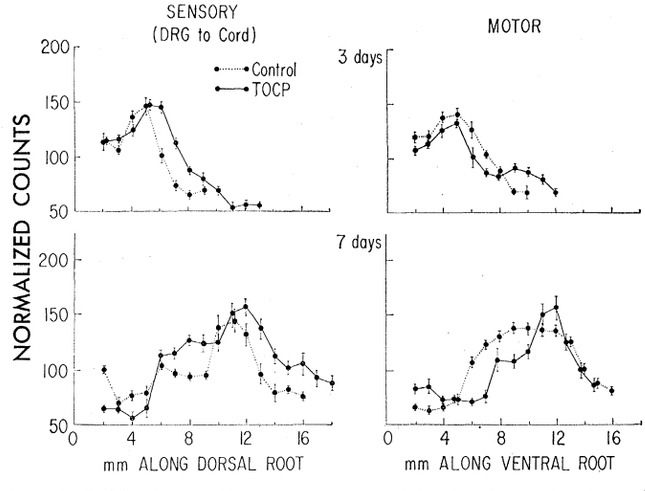


Fig. 1 (left). Autoradiograms were used in the determination of the distribution of H^3 in dorsal (sensory) and ventral (motor) roots of normal cats and cats made neuropathic by treatment with acrylamide at intervals after intraperitoneal injection of H^3-L-leucine. Results in representative cats 1, 3, 5, or 7 days after receiving the tracer are given. Maximum radioactivity was displaced away from the center of the dorsal root ganglion or anterior horn at about 1½ mm/day in normal cats but not in most cats treated with acrylamide. Points represent grain counts per field (0.01 mm^2) within a 1-mm segment of root (mean of eight determinations in each case) expressed as percentage of mean counts per 0.01 mm^2 in the entire root. Vertical lines represent ±1 standard error of the mean. DRG, dorsal root ganglion. Fig. 2 (right). Effect of TOCP on distribution of radioactivity along ventral and dorsal roots of cat neural tissue. Results are expressed as in Fig. 1. No significant difference in distribution of radioactivity between normal and TOCP-treated cats was observed.

cats with TOCP neuropathy, and in 12 controls matched for age, initial weight, and conditions of handling. Each cat received an intraperitoneal injection of tritiated L-leucine (1.4 to 2.6 mc per kilogram of body weight, New England Nuclear). At intervals from 4 hours to 2 weeks later, they were anesthetized with pentobarbital, and killed by perfusion through the left ventricle first with saline and then with buffered 2 percent glutaraldehyde. Sections (5 μm) of a lumbosacral cord segment in continuity with its roots and ganglion were cut in a cryostat, mounted on glass slides, dipped in Kodak NTB-3 nuclear track emulsion, exposed for 2 or 4 weeks at 5°C, developed, and stained. The geometric centers of the anterior horn and of the ganglion were used as reference points from which respective distances along ventral and dorsal roots were measured. Fields containing no visible perineurium were selected, and silver grains were counted in 0.01-mm^2 areas at intervals of 0.25 mm along the roots on each of at least two slides prepared from each cat. To facilitate comparison within and between groups of cats, the counts were normalized by expressing them as a percentage of mean activity along each root. Curves expressing mean specific activity of 1-mm segments of nerve root ($\pm$ 1 S.E.) as a function of distance from nerve cell bodies are illustrated in Figs. 1 and 2.

One day after injection of tritiated leucine, the radioactivity in the ventral root of cats in all three groups was maximum adjacent to the spinal cord, whereas in the dorsal root maximum activity bordered the ganglion. In control cats, the peak radioactivity was displaced away from the cord in the ventral root at the rate of 1.6 $\pm$ 0.5 mm per day and away from the ganglion along the dorsal root at the rate of 1.3 $\pm$ 0.2 mm/day. Similar results were obtained with cats treated with TOCP; motor flow was 1.5 $\pm$ 0.4 mm/day and sensory flow was 1.5 $\pm$ 0.3 mm/day (with the exception that, in one cat killed 2 days after it received the isotope, maximum radioactivity remained adjacent to cord and ganglion). In contrast, in all seven cats treated with acrylamide and killed 2 or more days after receiving the isotope, maximum radioactivity remained at the border of the dorsal root ganglion in the dorsal root and, in five of the seven, at the edge of the spinal cord in the ventral root. In the other two acrylamide-treated cats, killed 3 and 7 days after receiving tritiated leucine, a distribution of radioactivity along the ventral roots similar to that found in control animals was obtained (Fig. 1).

We could not detect a flow rate of less than 0.1 mm/day or more than 100 mm/day. Systemic administration of the tritiated amino acid increased the background and decreased the sensitivity of the method, because Schwann cells, fibroblasts, and, to some extent, axons themselves, can incorporate leucine into protein (*3*). In 5-μm longitudinal sections of roots, radioactivity in Schwann cells and fibroblasts could not be distinguished from that within the axons. In normal and neuropathic cats killed 4 hours after receiving the isotope, uptake of radioactivity was uniform along the entire length of ventral and dorsal roots. Thus, no gradient in uptake of leucine by cells along the roots seemed to occur. Differences in rate of absorption from the peritoneal cavity probably did broaden the peaks observed and contributed to a variation in the absolute size of the peaks.

Although glutaraldehyde fixation binds free amino acids to macromolecules (*4*), this should not have introduced a significant error in cats killed a day or more after they had received radioactive leucine; by 24 hours essentially all leucine would have been metabolized (*5*). To verify this point, we perfused two controls with formaldehyde, which does not bind amino acids to tissue elements (*4*). No significant differences in flow rate or in ratio of peak height to average root activity were noted.

We have confirmed the existence of a protein fraction moving along axons from motor and sensory neurons at about 1½ mm/day. Evidence of such transport was absent in most of the cats made neuropathic by acrylamide, but was present in cats given TOCP. The axonal degeneration seen in acrylamide-treated cats could result from this alteration in axoplasmic flow, but another mechanism would have to be postulated for the similar lesions induced by TOCP.

The disappearance of a migrating peak along ventral and dorsal roots in acrylamide neuropathy may be explained in either of two ways. (i) Formation of the proteins destined for transport may be blocked. Administration of puromycin, an inhibitor of protein synthesis, before administration of the radioactive amino acid causes a diminution in the amount of labeled protein appearing in nerve roots (*5*). (ii) Alternatively, owing to an abnormality in the transport mechanism itself, labeled proteins that are formed may not be transported normally within axons. The accumulation of radioactivity in segments of roots close to the cell bodies of origin in cats treated with acrylamide suggests that protein synthesis has occurred and that the defect is in the transport process itself.

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Electroconvulsive Shock Effects on a Reactivated Memory Trace: Further Examination

Abstract. *Rats showed amnesia for conditioned fear training if given an electroconvulsive shock immediately after training. Retention was unimpaired, however, when the electroconvulsive shock treatment was given 1 day after training immediately after the presentation of the stimulus used in the fear conditioning training. These results support the view that electroconvulsive shock disrupts memory trace consolidation but does not disrupt a recently reactivated memory trace.*

Electroconvulsive shock (ECS) produces retrograde amnesia (*1*). The evidence from numerous ECS studies indicates that retrograde amnesia is obtained only if the treatment is administered shortly (that is, within a few hours) after training.

Misanin *et al.* (*2*) reported results suggesting that memory is disrupted by ECS given 24 hours after training, pro-