ULTRASTRUCTURAL STUDIES OF THE DYING-BACK PROCESS
IV. DIFFERENTIAL VULNERABILITY OF PNS AND CNS FIBERS IN EXPERIMENTAL CENTRAL-PERIPHERAL DISTAL AXONOPATHIES

PETER S. SPENCER, Ph.D.

AND

HERBERT H. SCALAUMBURG, M.D.

(BRONX, N.Y.)

ABSTRACT

A companion paper in this issue (46) described the evolution of peripheral nervous system dying-back disease of the giant axonal type in animals chronically intoxicated with the neurotoxic hexacarbons n-hexane (CH₃CH₂CH₂CH₂CH₃), methyl n-butyl ketone or MBK (CH₃COCH₂CH₂CH₂CH₃), and 2,5-hexanedione (CH₃COCH₂CH₂COCH₃). The present study compares the distribution and pattern of peripheral (PNS) and central nervous system (CNS) dying-back disease produced by these three neurotoxic hexacarbons with that produced by acrylamide (CH₂CHCONH₂), and, in addition, employs these compounds to address unresolved issues in the dying-back process.

In the PNS, large myelinated fibers in tibial nerve branches supplying calf muscles were especially sensitive in rats intoxicated with hexacarbons. These nerve branches and sensory plantar nerves in the hindfeet were equally vulnerable in acrylamide-treated rats. In both conditions, fibers located at these sites commenced degeneration before the distal regions of much longer and smaller diameter nerve fibers in nerve branches supplying the flexor digitorum brevis muscle and, in rats intoxicated with hexacarbons, before equivalent regions of plantar sensory branches to the digits. Pacinian corpuscles sited in the hindfeet of intoxicated cats were much less vulnerable to MBK than to acrylamide. Rats and cats intoxicated with hexacarbons displayed giant axonal swellings in vulnerable regions of the PNS concurrently with similar swellings in sensitive regions of the CNS. CNS degeneration in these animals was accompanied by pronounced endoneurial edema.

In the CNS, rostral regions of long, ascending tracts (dorso-spino-cerebellar, gracile and, later, the cuneate) and the caudal end of long, descending tracts (lateral columns, ventrolateral and ventromedial tracts) of hexacarbon-treated animals were especially vulnerable. After prolonged intoxication of cats with MBK, giant axonal swelling was also found in preterminal and terminal axons in Rexed laminae V-VII at spinal levels C₄ through S₃. Neurofilament proliferation without giant axonal swelling was seen in CNS fibers of rats intoxicated with acrylamide.

From the Departments of Pathology and Neuroscience, the Saul R. Korey Department of Neurology, and the Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, The Bronx, New York 10461.
Taken in concert, the findings underline the importance of axon diameter and length in determining the hierarchy of fiber vulnerability and indicate the common sensitivity of selected regions of the PNS and CNS. The term central-peripheral distal axonopathy is introduced to emphasize the widespread, distal distribution of disease in these and in similar experimental conditions. It is suggested that certain human neuropathies (toxic, nutritional, uremic, diabetic and some hereditary polyneuropathies, and the neuropathy associated with multiple myeloma) are additional examples of central-peripheral distal axonopathies.

INTRODUCTION

The term dying-back was introduced to describe the spatio-temporal pattern of central and peripheral nerve fiber pathology in degenerative diseases such as Friedreich's ataxia (9, 12, 21). The pathology of dying-back diseases, discussed in detail in the companion paper (46), is characterized by an early, distal axonal degeneration and, with time, a slow, proximal spread of nerve fiber breakdown. It was suggested (9, 10) that in the dying-back process, there was a malfunction of neuron cell bodies which resulted in a gradual decrease in the production of materials required for the maintenance of axonal integrity. On the basis of this theory, the most distal extremities of the axons would suffer from a reduction of essential materials exported from the neuronal perikaryon since proximal regions would be supplied first. Axonal degeneration would commence at the terminals of the longest and largest axons, and show a temporal, seriate progression of change proximally, pari passu with increasing perikaryal impairment.

Studies by Cavanagh and others (5, 7, 8, 10, 28), on the effects of organophosphorus compounds on chickens and cats, clearly established that certain toxic chemicals could be utilized to produce animal models of dying-back disease. Although experimental studies with tri-orthocresyl phosphate (TOCP) (5, 11, 28), acrylamide (17, 26) and isoniazid (41) had produced morphologic evidence which appeared to support the classical concept of the pathogenesis of the dying-back process, Prineas (34, 35) pointed out that the observed pattern of degeneration produced by TOCP and by acrylamide could be explained without invoking a theory of neuron perikaryal disturbance. A more recent study using acrylamide further challenged the underlying assumptions of the classical concept of the dying-back process (40, 43): first, it was shown that the longest and largest fibers were not more vulnerable than equivalent regions of shorter or smaller fibers and, second, it was found that axonal change commenced in a distal, multifocal distribution proximal to the axon terminal. A similar distal, nonterminal, multifocal distribution of early axonal change was seen in experimental isoniazid intoxication

These three hexacarbons, and to a lesser extent acrylamide (CH₂CHCONH₂), offer important advantages for the further study of the dying-back process since the initial change of axon neurofilament accumulation is accompanied by clearly visible focal fiber swellings and secondary changes in the myelin sheath. These compounds are employed in the present study to address some of the unresolved issues in the dying-back process: 1) The pattern and distribution of beginning nerve fiber pathology produced by these two types of compounds; 2) the vulnerability of selected nerve fibers in certain regions of the peripheral nervous system (PNS) and of the central nervous system (CNS) and, 3) the clinically inapparent equal sensitivity of the PNS and CNS, a finding which has led to the designation central-peripheral distal axonopathy.

MATERIALS AND METHODS

Twelve young, adult cats and 62 young, adult, Sprague-Dawley rats were used for this study. All animals, with the exception of six rats, were housed in smooth-floored cages to prevent trauma to plantar nerves during activity. Animals were intoxicated either by inhalation, subcutaneous injection or per os. Each animal was periodically weighed and examined for signs of physical or neurological deterioration.

Eight cats received subcutaneous injections of 150 mg/kg of undiluted methyl n-butyl ketone or MBK (99.66% pure), twice daily, 5 days a week for up to 24 weeks. Injection sites were rotated over the back to minimize skin ulceration. Four control cats received twice daily, subcutaneous injections of a similar volume of saline (0.2 mL/kg). Tissue was obtained both by biopsy and after systemic perfusion with fixatives. Anesthetized cats underwent hindfoot plantar biopsies after 45 and 135 days of continuous intoxication. Tissue was obtained first from right hindfeet and, on the second occasion, from left hindfeet. Biopsied tissues included pacinian corpuscles from the central toepad, slips of flexor digitorum brevis muscle and branches of the lateral plantar nerve. These tissues were immersed in phosphate-buffered 5% glutaraldehyde for 1-3 hours (pH 7.4).

Rats were each exposed to one of four compounds: n-hexane, MBK, 2,5-hexanodione or acrylamide. n-Hexane (98.98% pure) was administered to 11 animals either by inhalation of 400-600 p.p.m. continuously for 2 to 23 weeks or by subcutaneous injection of 650-2000 mg/kg/d, 5 days a week for up to 35 weeks. MBK was administered to 11 animals by inhalation of 600 p.p.m. for 212 hours continuously, or of 1300 p.p.m. 6 hours a day, 5 days a week for up to 17 weeks. 2,5-Hexanodione (Eastman) was given to 14 animals either by oral ingestion or by subcutaneous injection (220-520 mg/kg/d) for 8 to 19 weeks. Acrylamide dissolved in saline was administered by subcutaneous injection to 10 animals (10 to 60 mg/kg/d for 4 to 40 days). Sixteen age and weight-matched rats were used as controls.

For perfusion, animals were anesthetized with sodium pentobarbitone containing heparin and perfused through the aortic arch with 4% paraformaldehyde (30 s) followed by 5% glutaraldehyde (15 min), each in 0.1 M phosphate buffer (pH 7.4). After perfusion, tissue was removed from the peripheral and central nervous systems of intoxicated and control animals. The PNS was sampled as follows: in cats intoxicated with MBK for 2, 4 or 6 months, tissue was taken from multiple sites of the sciatic, tibial, and plantar nerves in the hindleg. Pacinian corpuscles in the hindfeet, corresponding lumbar spinal roots and dorsal root ganglia, and hindlimb muscles (gastrocnemius, tenuissimus, interosseus and lumbral); in rats intoxicated with hexacarbons or acrylamide, the sciatic, tibial and plantar nerve complex, plus attached gastrocnemius, lumbral and flexor digitorum brevis muscles were removed in continuity from each hindlimb. The tissue from one hindlimb was processed intact and, from the other hindlimb, segments were removed from multiple levels of the sciatic nerve, the tibial and plantar nerves, tibial nerve branches supplying the calf muscles and plantar nerve branches supplying flexor digitorum brevis. CNS tissue was sampled as follows: in cats intoxicated with
MBK, up to six, widely-separated levels from the entire length of the spinal cord and medulla oblongata and, in rats intoxicated with n-hexane, MBK or 2,5-hexanediol, multiple levels of the spinal cord, the medulla oblongata and the cerebellum.

Lengths of perfused nerve and muscle in continuity plus PNS and CNS tissue segments and fixed biopsy tissue, were immersed for 1–3 hours in 2% Dalton's chrome osmium solution, dehydrated stepwise, immersed in propylene oxide or acetone, and infiltrated with epoxy resin. The lengths of rat nerve and muscle in continuity were used for the preparation of individual nerve fibers teased from the distal ends of tibial and plantar nerve branches. Sampled nerves included: 1) branches of the medial planar nerve supplying the flexor digitorum brevis muscle; 2) neighboring sensory branches to the digits; 3) the tibial nerve branch to flexor hallucis longus, tibialis posterior and flexor digitorum longus; 4) the tibial nerve branch supplying the medial head of gastrocnemius, and 5) the tibial nerve branches supplying plantaris, soleus and the lateral head of gastrocnemius (20). Individual fibers, up to 3 cm long, were removed from each nerve branch, placed on a slide and hardened by heat, provided with a coverslip, and examined with the light microscope. The small segments of tissue were placed in molds containing epoxy resin and hardened by heat. One micrometer epon sections, cut from tissue blocks, were stained with toluidine blue and examined by light microscopy. Thin sections of selected areas were stained with uranyl acetate followed by lead citrate, and examined with the electron microscope.

RESULTS

Clinical Assessment

A full description of the clinical findings in rats intoxicated with n-hexane, MBK and 2,5-hexanediol was given in a companion report (46). Briefly, these animals slowly developed symmetrical, hindlimb weakness with foot-drop and, after prolonged intoxication, concurrent weakness of the forelimbs (Fig. 1). Rats intoxicated with acrylamide developed an unsteady, shaky gait after 30–40 days of 20 mg/kg/d, after 12–15 days of 40 mg/kg/d and after 8 days of 60 mg/kg/d. These animals were sacrificed before they developed frank hindlimb weakness.

Cats intoxicated with MBK frequently appeared narcotized for a few hours following each injection. Soon after injection, excessive salivation was noted and this sometimes was continuous. Three animals became systemically ill, weakened and subsequently died. Three other cats developed signs of neuropathy after 8–10 weeks of intoxication with 300 mg/kg/d. At this time, affected animals displayed weakness of the hindquarters which resulted in a crouching, unsteady gait and which progressed to a severe hindlimb foot-drop by 10–12 weeks. By 16 weeks, these cats were unable to walk and dragged themselves with weakened forelimbs. Cats which had received saline injections for a similar period appeared normal and gained weight more rapidly than cats treated with MBK.

Pathology of Intoxicated Animals

The principal features of PNS and CNS damage produced by the three hexacarbons and by acrylamide are summarized in Tables 1 and 2.

Rat PNS Pathology: A detailed description of the evolution of murine peripheral giant axonal degeneration produced by n-hexane, MBK or 2,5-
hexanedione was given in a companion study (46). Briefly, all three compounds produced an identical pattern of distal nerve fiber change which consisted of multifocal, giant axonal swellings (initially on the proximal sides of paranodes), paranodal demyelination and remyelination, fiber degeneration and regeneration. Fine structural highlights included granular axonal mitochondria, honeycombed interdigitated Schwann cell/axon networks, corrugated myelin sheaths, and prominent accumulations of 10 nm neurofilaments. Nerve fiber degeneration was accompanied by a variable degree of endoneurial edema.

Rats intoxicated with acrylamide revealed similar, but smaller distal, multifocal axonal swellings sited on the proximal sides of nodes of Ranvier. These were rarely associated with paranodal demyelination. The segmental remyelination reported by Hopkins (26) was not encountered. Complete fiber breakdown consisting of chains of ovoids was a common feature. Sometimes, these chains of ovoids were continuous proximally with preserved portions of the fiber. Electron microscope study revealed accumulations of 10 nm neurofilaments.
TABLE 2
Pathology and Distribution of CNS Damage in Cats (except where noted*)

<table>
<thead>
<tr>
<th>Hexacarbon</th>
<th>Acrylamide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histological Features</strong></td>
<td></td>
</tr>
<tr>
<td>Scattered giant axonal swellings</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Fiber breakdown late</td>
<td>Characteristic</td>
</tr>
<tr>
<td><strong>Ultrastructural Features</strong></td>
<td></td>
</tr>
<tr>
<td>10 nm neurofilament proliferation in axons</td>
<td>Pronounced</td>
</tr>
<tr>
<td>Honeycomb, interdigitated oligodendroglial/neuron profiles</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td></td>
</tr>
<tr>
<td>Distal in long ascending and descending tracts initially</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Proximal in long ascending and descending tracts later</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Involvement of shorter tracts later</td>
<td>Characteristic</td>
</tr>
<tr>
<td>Involvement of cerebellar vermis</td>
<td>Early in white matter (not*)</td>
</tr>
<tr>
<td><strong>Involvement of modular</strong></td>
<td></td>
</tr>
<tr>
<td>Dorsal spinocerebellar tracts</td>
<td>Early</td>
</tr>
<tr>
<td>Ventro-lateral (vestibulospinal?)</td>
<td>Early</td>
</tr>
<tr>
<td>Pyramidal tracts</td>
<td>Early</td>
</tr>
<tr>
<td>Gracile tracts and nuclei</td>
<td>Early</td>
</tr>
<tr>
<td>Cuneate tracts and nuclei</td>
<td>Late</td>
</tr>
<tr>
<td><strong>Involvement of spinal cord</strong></td>
<td></td>
</tr>
<tr>
<td>Ascending Tracts</td>
<td></td>
</tr>
<tr>
<td>Dorsospino-cerebellar tracts</td>
<td>T6 and rostrally</td>
</tr>
<tr>
<td>Gracile tracts</td>
<td>Frequent at C1-C6</td>
</tr>
<tr>
<td>Cuneate tracts</td>
<td>C1 only</td>
</tr>
<tr>
<td>Descending Tracts</td>
<td></td>
</tr>
<tr>
<td>Lateral columns (corticospinal &amp; vestibulospinal?)</td>
<td>C4, increasing in severity to L6</td>
</tr>
<tr>
<td>Ventrolateral and ventromedial (vestibulospinal &amp; reticulospinal?)</td>
<td>C4, increasing in severity to L6</td>
</tr>
<tr>
<td><strong>Spinal Gray</strong></td>
<td></td>
</tr>
<tr>
<td>Rexed laminaae V through dorsal VII</td>
<td>Preterminal and terminal changes at C4-S2</td>
</tr>
<tr>
<td></td>
<td>C6-L6</td>
</tr>
</tbody>
</table>

filaments, abnormal mitochondria and honeycombed, interdigitated Schwann cell/axon networks.

**Rat PNS Distribution of Damage**: The spatio-temporal hierarchy of nerve fiber vulnerability was determined for each group of intoxicated animals by sampling fine nerve branches of tibial and plantar nerves in the hindlimbs of animals (Fig. 2) before the development of clinical signs. Rats intoxicated with n-hexane, MBK or 2,5-hexanedione displayed the following decreasing order of peripheral nerve fiber vulnerability: First (most vulnerable), the branches of the tibial nerve supplying the calf muscles (Fig. 3, 4); second, the plantar nerve branches supplying the flexor digitorum brevis muscle, and

**Fig. 3.** Light micrograph of a transverse section of the tibial nerve (left) and one of its branches to the calf muscles (right) beneath the popliteal fossa in a normal rat. Note the large size of myelinated fibers in the branch to the calf muscle (right) relative to that of fibers in the posterior tibial nerve (left) supplying the foot. This figure and Fig. 4-20 are one micrometer epoxy sections stained with toluidine blue. × 150.

**Fig. 4.** Similar region to Fig. 3 taken from a rat with giant axonal neuropathy. The posterior tibial nerve (left) shows little evidence of change except for perivascular endoneurial edema (arrow). The branches to the calf muscles (right) display extensive endoneurial edema and a few fibers which have undergone giant axonal swelling. MBK. × 150.

**Fig. 5.** Tibial nerve removed from a rat with severe neuropathy. The posterior tibial nerve fascicle (left two thirds) contains several swollen fibers and a degree of endoneurial edema. The fascicles supplying the calf muscles (right third) show evidence of extensive fiber loss and marked endoneurial edema. 2,5-Hexanedione. × 150.
third, (least vulnerable), adjacent levels of sensory plantar nerve branches innervating the digits. With time, axonal degeneration ascended the plantar nerves and was seen in the tibial nerve trunk up to the level of the exit of the branches to the calf muscles, while the degeneration in these branches ascended pari passu toward the sciatic notch (Fig. 5, 6). After prolonged intoxication, these two independent, ascending waves of tibial and plantar nerve degeneration contributed to the overall damage seen in the sciatic nerve (Fig. 7). At this time, L4 and L5 ventral roots were slightly affected at the level of the dorsal root ganglion, but not at their entry into the spinal cord. Spinal ganglion neurons usually were spared. Corresponding dorsal roots contained swollen fibers close to the spinal cord, but not at the level of the dorsal root ganglion (Fig. 8–10). Anterior horn cells located in the lumbosacral spinal cord appeared normal in one micrometer epon sections (Fig. 11). Motor nerve terminals innervating extrafusal fibers of sampled muscles were either normal or abnormally swollen (Fig. 12), at a time when giant axonal swellings were commonly found in the distal regions of nerve fibers supplying these muscles.

Rats intoxicated with acrylamide displayed a different distribution of beginning peripheral nerve damage. The following decreasing order of fiber vulnerability was determined: First (most vulnerable), the branches of the tibial nerve supplying the calf muscles and the plantar sensory nerves supplying the digits, and second (less vulnerable), the plantar nerve branches supplying the flexor digitorum brevis muscle.

Examination of tibial and plantar nerve branches in unexposed rats showed that the largest myelinated nerve fibers were located in the tibial nerve branches to the calf muscles. Myelinated fibers in the tibial nerve to the foot and in the plantar nerves were consistently of smaller diameter (Fig. 3). Control animals which had lived in cages with wire-mesh floors consistently displayed abnormal plantar nerves and, in severe cases, some pathology in posterior tibial nerves. Multiple, granular axonal mitochondria (45) were commonly seen. Tibial nerve branches supplying calf muscles were normal in such animals.

Cat PNS Pathology: A total of 60 pacinian corpuscles were removed from the hindpaw toe pads of three MBK-treated cats on each of three occasions, two at biopsy and one after perfusion. After 6 weeks of intoxication, most corpuscle axon terminals appeared normal (42). One animal, with a severe gait impairment after 19 weeks of MBK treatment, had corpuscle axon terminals which were normal or which displayed 10 nm neurofilament accu-

---

**Fig. 6.** A more proximal level of the nerve illustrated in Fig. 5. Note how the fibers supplying the calf muscles (right portion) are clearly demarcated by differential endoneurial edema from the portion of the tibial nerve supplying the foot (left). 2,5-Hexanedione. × 130.

**Fig. 7.** Sciatic nerve of a rat with severe neuropathy. Many fibers display giant axonal swelling. These are distributed homogeneously throughout the nerve. When this nerve was traced distally, swollen fibers were found to exit in the branches to the calf muscles. MBK. × 140.

**Fig. 8.** Lower lumbar dorsal root ganglion (left) and attached ventral root from a rat with severe neuropathy. Note the absence of giant axonal swelling in the dorsal root compartment and the presence of a few swollen fibers (arrow) in the ventral root. 2,5-Hexanedione. × 140.
mulation. After 17 weeks of intoxication of another animal, who was unable to walk at the time of biopsy, 6 corpuscles were denervated, 2 showed severe axonal damage, 6 displayed neurofilament accumulation and none was normal. In one asymptomatic animal, pacinian corpuscles in the hindfoot toepad were intact when axonal swelling, demyelination and remyelination were present in the posterior tibial nerve twigs supplying the calf muscles.

Cats with MBK neuropathy displayed evidence of degeneration and regeneration in plantar nerves. Sciatic nerves contained giant axon swellings and stretches of demyelination and early remyelination. Proximal and distal muscles contained fibers and motor nerve terminals which were either unaffected or swollen.

**Cat CNS Distribution of Damage:** Central nerve fiber changes in cats treated with MBK are listed in Table 1 where they are compared with previous reports (35, 40, 43) of CNS damage in cats intoxicated with acrylamide. These two types of compounds produced a similar distribution of tractal damage: In MBK-treated animals, rostral regions of long, ascending tracts and caudal regions of long, descending tracts displayed the earliest changes in the form of giant axonal swellings. In more severely affected cats, these same regions showed less swelling, but more advanced nerve fiber degeneration; concurrently, more proximal regions of these tracts displayed evidence of early nerve fiber damage in the form of giant axonal swellings. Preterminal and terminal axons of long, descending tracts located in the spinal gray were enlarged. Degeneration of boutons terminaux abutting spinal neurons, previously reported in cats poisoned with TOCP (28), was also seen (Fig. 13).

**Rat Distribution of Damage:** The presence of giant axonal swelling in animals intoxicated with hexacarbons readily permitted the identification of early nerve fiber pathology. Swollen axons were present in ventro-lateral and dorso-lateral tracts, the gracile nuclei of the medulla oblongata and the white matter of the cerebellar vermis (Fig. 14, 15). These changes were present before clinical hindlimb weakness was apparent and were concurrent with beginning giant axonal degeneration in the most vulnerable regions of the PNS. When neuropathy was pronounced, degenerating fibers were found in the rostral regions of gracile tracts. Axonal swellings were also detected in dorsal and lateral columns and in ventro-medial quadrants of the lumbosacral spinal cord (Fig. 16–20).

The absence of such distinctive evidence of early axonal damage in rats treated with acrylamide made a comparable assessment of the distribution of nerve damage impossible.

**CNS Pathology:** Light microscope observation of tissue obtained from rats

---

**Fig. 9.** A section of the same ventral root illustrated in Fig. 8 taken near its junction with the spinal cord. There is no evidence of giant axonal swelling. 2,5-Hexanediene. × 180.

**Fig. 10.** Lower lumbar dorsal root of a rat taken close to its junction with the spinal cord. Numerous fibers display giant axonal swelling (arrow). 2,5-Hexanediene. × 180.

**Fig. 11.** Rat with severe neuropathy. Lower lumbar spinal cord anterior horn displaying motor neurons of normal appearance. MBK. × 370.

**Fig. 12.** Extrafusal motor nerve terminals in the gastrocnemius muscle from a rat with severe neuropathy. One of the terminals (arrow) is markedly swollen. 2,5-Hexanediene. × 1,480.
and cats treated with hexacarbons revealed abnormally large fibers with swollen axons and myelin sheaths inappropriately thin for their axon diameter. These swollen axons initially appeared darkened and glassy, and contained dark blue granules. More severely damaged fibers exhibited light and dark-blue staining axons and, sometimes, evidence of complete fiber breakdown. Ultrastructural examination of this tissue revealed nodal, internodal and terminal axonal swellings containing accumulations of 10 nm neurofilaments, tubulo-vesicular profiles, mitochondria and dense lamellar profiles (Fig. 21–23). Myelinated fibers exhibiting giant axonal swelling contained whorled masses of neurofilaments and segregated neurotubules (Fig. 22). Some fibers showed adaxonal oligodendrocyte invagination and honey-combed, interdigitated oligodendrocyte/axon networks. A similar pattern of pathology was found in animals treated with acrylamide, although the degree both of neurofilament accumulation and axonal swelling was less marked.

**DISCUSSION**

The present study in concert with the companion paper (46), demonstrate that the nervous system disease produced by neurotoxic hexacarbons (n-hexane, methyl n-butyl ketone and 2,5-hexanediol) and that produced by acrylamide have many clinical and pathological features in common. Both types of compounds produce distal, symmetrical neuropathy associated with multifocal, neurofilamentous accumulations in a distal and slowly ascending distribution in vulnerable areas of the PNS and CNS. These findings confirm earlier reports (39, 44, 47) that the neurotoxic hexacarbons produce nervous system disease of the dying-back type.

A cardinal finding in animals with subclinical hexacarbon or acrylamide neuropathy is that the distal, non-terminal regions of proximal tibial nerve branches supplying the hindlimb calf muscles display evidence of disease before equivalent regions of plantar nerve fibers supplying muscles in the hindfeet. The early involvement of proximal tibial nerve fibers accounts for the report of Saída, Mendell and Weiss (38) who found abnormalities in the sciatic nerve of MBK-treated rats before they appeared either in the nerve roots or in the intramuscular nerves and terminals of interosseous muscles. One must presume that their failure to examine the spatio-temporal evolution of pathology throughout the sciatic/tibial/plantar nerve complex and involved regions of the CNS, coupled with their curious expectation of pathology in sensory and motor neurons supplying the hindfeet, led these authors to the erroneous conclusions that MBK produced a fundamentally different type of pathology from that produced by acrylamide, and that the former could not be classified as a neuropathy of the dying-back type. Another study (30) from

**Fig. 13.** Rexed layer dorsal VII of the sacral spinal cord of a cat with severe MBK neuropathy. Many neurons and their dendrites are associated with markedly swollen terminals (arrows). × 270.

**Fig. 14.** Spinocerebellar tract in the medulla oblongata from a rat with neuropathy. Numerous giant axonal swellings are visible. 2,5-Hexanediol. × 480.

**Fig. 15.** Anterior vermis from an animal with severe neuropathy. The white matter contains a severely swollen myelinated fiber (s). 2,5-Hexanediol. × 400.
the same laboratory, of anterograde fast axonal transport in MBK-treated rats, has revealed a progressive proximal decrement correlating with degree of clinical impairment which is consistent with the dying-back phenomenon.

The present study has demonstrated that the distal regions of nerve fibers supplying the calf muscles are more vulnerable than equivalent regions of much longer nerve fibers supplying the muscles in the feet of rats intoxicated with hexacarbons or with acrylamide. The nerves to the calf muscles contain the largest myelinated fibers in the hindlimb while the fibers supplying the hindfoot are considerably smaller in diameter (6). It seems apparent, therefore, that fiber diameter is more important than axon length in determining the differential vulnerability of peripheral nerve fiber degeneration in these experimental dying-back diseases. Whether fiber diameter is more important than axon length in the CNS component of these dying-back diseases has not been determined. Distal axonal degeneration certainly occurred in some of the largest, ascending and descending fibers in the CNS. It is evident that axon length has a role in determining CNS nerve fiber vulnerability in hexacarbon-treated animals since degeneration began earlier and was more severe in the gracile tracts than in adjacent levels of the cuneate tracts, a pattern also noted in animals intoxicated with TOCP and with acrylamide (3, 7, 10, 34, 35).

This study has also revealed some differences between the distribution of beginning peripheral nerve fiber change produced by the three hexacarbons and that produced by acrylamide. The plantar sensory nerves were much more vulnerable to acrylamide than to the hexacarbon compounds. This is consistent with our previous finding (40) that certain sensory nerve terminals in the hindfeet of cats, such as pacinian corpuscles, were more vulnerable to acrylamide than adjacent motor nerve terminals. By contrast, pacinian corpuscles in the hindfeet of cats intoxicated with MBK showed a remarkable resistance to damage and degenerated long after hindlimb weakness was profound. The special vulnerability of the nerves supplying the calf muscles in the hexacarbon neuropathies might account for the clinical findings of distal weakness and loss of Achilles reflex concurrent with sensory abnormalities confined to the feet in patients exposed to \( n \)-hexane or to MBK (2, 24). TOCP produced a similar clinical picture more rapidly (50, 51), although studies of cats intoxicated with this compound demonstrated that the deep branch of the common peroneal nerve (not examined here) was more vulnerable than tibial nerve branches to the lateral head of the gastrocnemius (8). A consistent clinical feature in the present series of experiments (40, 46, 48) has been the striking contrast in gait between animals intoxicated with acrylamide and those treated with neurotoxic hexacarbons. Rats and cats intoxicated

---

**Fig. 16-19.** Progressively descending transverse sections of the gracile tracts of the spinal cord of a rat with severe neuropathy. 16. Severe degeneration at the level of medulla oblongata. 17. Degeneration and some swollen axons at C4. 18. Degeneration and some axonal swelling at C7. 19. Pronounced giant axonal swelling at L4 with little advanced degeneration. 25-Hexanediene. x 150.

**Fig. 20.** Gracile (left) and cuneate (right) tracts in the medulla oblongata of a cat with severe MBK neuropathy. Note the advanced degeneration in the gracile tract and the prominent giant axonal swellings in the cuneate tract. x 560.
with hexacarbons demonstrated a stereotyped picture of progressive hindlimb weakness which, with time, evolved into an unsteady gait with foot-drop. By contrast, animals intoxicated with acrylamide initially developed an unsteady, weaving gait accompanied by head titubation before hindlimb weakness and foot-drop became evident, making them readily distinguishable from animals intoxicated with hexacarbons. It is not clear whether this graphic clinical phenomenon in acrylamide-treated animals is related to the morphological demonstration (40) of vulnerability of sensory nerve fibers supplying intrafusal muscles or if there is an early effect on brainstem-cerebellar pathways. The physiological study of Sumner and Asbury (49) would seem to implicate the muscle spindle, while that of Kuperman (29) emphasized the presence of physiological abnormalities in the CNS.

Another cardinal finding, drawn from the hexacarbon studies, is that giant axonal change is an early feature of degeneration concurrently in both the PNS and the CNS. This observation, together with previous findings of CNS degeneration in cats intoxicated with acrylamide (18, 35, 43), indicate that the dying-back process commences contemporaneously in certain vulnerable fibers, irrespective of their location either in the PNS or CNS. Although Cavanagh and his colleagues (9, 11, 28) also demonstrated a similar distribution of concurrent PNS and CNS degeneration in the experimental dying-back disease produced by TOCP, the importance of this observation has been poorly appreciated, presumably because the human clinical picture produced by many of these neurotoxins is dominated by signs and symptoms attributable solely to PNS damage. Initially, these PNS signs may mask the central nervous system disease and, later, emerge when the PNS is allowed to recover (39). To emphasize this combination of concurrent PNS and CNS degeneration, we propose that the term central-peripheral distal axonopathy be adopted to identify diseases showing this pattern. This designation would presently encompass such neuropathies as those produced by organophosphorus compounds, acrylamide, n-hexane and MBK. However, it seems likely that distal, symmetrical human neuropathies other than those produced by these neurotoxic chemicals, upon inspection will prove to be central-peripheral distal axonopathies (45). Some potential candidates for this appellation include nutritional (beri-beri, pellagra, alcoholic), uremic, diabetic, some of the hereditary polyneuropathies, and the neuropathy associated with multiple myeloma. CNS degeneration, most especially involving the posterior columns, has been described in all these conditions (1, 4, 12, 13, 16, 19, 22, 23, 25, 32, 33, 36, 37, 52–56; see also 14, 15, 31). This has usually been attributed either to local CNS disease, or to damage of spinal ganglion cells secondary to ascending PNS damage, although it was recently demonstrated that selective

**Fig. 21.** Nodal giant axonal swelling in the gracile nucleus of a rat with neuropathy. This figure and Fig. 22, 23 are thin epoxy sections stained with uranyl acetate and lead citrate. 2,5-Hexanediene. × 6,250.

**Fig. 22.** Giant axonal swelling (s) located in the medulla oblongata of a rat with neuropathy. Some of the neurotubules have been sequestered in one area (arrow). MBK. × 9,400.

**Fig. 23.** Giant axonal swelling of the nerve terminal (t) synapsing with a dendrite (d) of a neuron located in the medulla oblongata of a rat with neuropathy. 2,5-Hexanediene. × 9,200.
vascular damage to human peripheral nerve is not accompanied by degeneration of the gracile tracts (31). In the light of this observation and those of the present study, it is likely that the CNS findings in the above conditions represent the central components of central-peripheral distal axonopathies.

Acknowledgements: The authors thank W. Krasavage, Drs. G. DiVincenzo, A. N. M. Nasr, J. O’Donoghue, R. L. Raleigh and C. J. Terhaar of the Health and Safety Laboratory of Eastman Kodak Company, Rochester, New York for discussion and help with the intoxication of animals by inhalation of MBK, Dr. R. D. Terry for encouragement, Professor P. K. Thomas and Dr. A. Aguayo for discussion, Monica Bischoff, Lynn Carmickle, Howard Finch, Miriam Pakingan and Everett Swanson for technical assistance, and Elaine DelPavero for assistance in the preparation of the manuscript.

This study was supported by a grant from the American Cyanamid Company, Dow Chemical Company, Vistron Corporation, Nalex Chemical Company, a grant from the Tennessee Eastman Company, a division of Eastman Kodak Company, and by U.S.P.H.S. grants OH 00595, NS 03556 and NS 08952.

Dr. Spencer is the recipient of a Joseph P. Kennedy, Jr. Fellowship in the Neurosciences.

REFERENCES

ULTRASTRUCTURAL STUDIES OF THE DYING-BACK PROCESS. IV. 319


