Experimental neuropathy produced by 2,5-hexanedione—a major metabolite of the neurotoxic industrial solvent methyl n-butyl ketone

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SYNOPSIS Chronic exposure of rats to 2,5-hexanedione ($\text{CH}_3\text{COCH}_2\text{CH}_2\text{COCH}_3$), a major metabolite of the neurotoxic industrial solvent methyl n-butyl ketone ($\text{CH}_3\text{COCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), has been shown to cause a clinical peripheral neuropathy with dying-back peripheral and central nervous system degeneration characterized by giant axonal swellings filled with neurofilaments. This pattern of disease is similar to that produced by methyl n-butyl ketone.

The industrial solvent methyl n-butyl ketone ($\text{CH}_3\text{COCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), has been shown to be the principal agent responsible for the recent outbreak of peripheral neuropathy in a fabric-impregnating plant in Columbus, Ohio. Neuropathy was produced in several species of experimental animal which, after chronic exposure to methyl n-butyl ketone (MBK) by various routes, developed progressive distal symmetrical weakness (Duckett *et al.*, 1974; Mendell *et al.*, 1974; Spencer *et al.*, 1975). The characteristic histopathological changes of focal axonal swelling, associated with accumulations of neurofilaments, were similar to biopsy observations in two cases of giant axonal neuropathy in children (Asbury *et al.*, 1972; Carpenter *et al.*, 1974) and in one case of glue-sniffing neuropathy attributed to the solvent n-hexane ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$) (Korobkin *et al.*, 1975) which has been shown to be neurotoxic (Ishii *et al.*, 1972). Furthermore, the distribution of the axonal disease in the MBK-treated animals, in the distal regions of long peripheral and central nerve fibres, corresponded to the dying-back pattern of fibre degeneration seen in some other experimental

toxic neuropathies (Cavanagh, 1964; Prineas, 1969; Schaumburg *et al.*, 1974).

Biochemical study of the metabolic fate of MBK in experimental animals revealed that a major, water-soluble metabolite of MBK was the compound 2,5-hexanedione ($\text{CH}_3\text{COCH}_2\text{CH}_2\text{COCH}_3$) (G. DiVincenzo and J. Dedinas, Eastman Kodak Company, personal communication). The present study demonstrates that 2,5-hexanedione is neurotoxic and that it causes a pattern of nervous system degeneration similar to that produced by MBK.

METHODS

Six 400 g Sprague-Dawley rats were injected subcutaneously five days a week with 0.1 ml of 2,5-hexanedione for 13 weeks plus 0.2 ml or 0.4 ml for 6-10 weeks—that is, up to a mean of 340 mg/kg/day. Animals were examined at weekly intervals and were killed before or soon after the onset of clinical signs. Each animal was anaesthetized with sodium barbitone containing heparin and perfused through the heart with 4% paraformaldehyde followed by 5% glutaraldehyde, each in a phosphate buffer (pH 7.4). Tissue was sampled as follows: from up to seven sites along the sciatic, tibial, and plantar nerves in the hindlimbs, the lumbosacral dorsal root ganglia and corresponding spinal roots, leg and hindfoot muscles, and the medulla oblongata.

Tissues were postfixed in 2% Dalton's chrome

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osmium, dehydrated, immersed in propylene oxide, and infiltrated with epoxy resin. Single nerve fibres, up to 2.5 cm long, were teased from proximal and distal sites in the tibial nerve. Longitudinal and transverse $1\ \mu\text{m}$ sections, cut from epoxy-embedded tissues, were stained with toluidine blue and examined by light microscopy. Thin sections of selected areas were stained with uranyl acetate and lead citrate, and examined by electron microscopy.

RESULTS

The onset of clinical signs was insidious, occurred symmetrically in the hindlimbs, and was characterized by a waddling gait in which the feet were everted and placed flat on the ground. Pathological changes were found in all animals studied irrespective of the presence or absence of clinical signs. Nerve fibre degeneration was most prominent in the distal portions of the tibial nerve and its branches, and was rarely encountered in the proximal regions of the sciatic nerve or in the spinal roots. Lumbosacral anterior horn cells and dorsal root ganglion cells appeared histologically normal. In the central nervous system, nerve fibre abnormalities in the form of swollen axonal profiles were most prominent in the gracile nucleus, although, in animals with early clinical neuropathy, changes were also found in the cuneate nucleus and in a superficial, ventrolateral zone best identified as the dorsal and ventral spinocerebellar tracts.

Teased myelinated fibres prepared from the tibial nerve and its branches displayed a spectrum of abnormality. The most proximal abnormalities were intercalated, remyelinated internodes, separating preserved portions of the fibre. Pronounced focal axonal swellings, some of which were sited proximal to a node of Ranvier and which were associated with paranodal demyelination were seen distally (Fig. 1). Further distally, severely corrugated internodes were encountered. These were interrupted by normal or abnormally lengthened nodal gaps or

FIG. 1 Portion of a teased fibre isolated from a branch of a posterior tibial nerve in the mid-calf. Note the giant axonal swelling sited proximal to a short, demyelinated region (d) and the distal corrugated myelin. $\times 200$. FIG. 2 Intercalated remyelinated internode (r) separating corrugated segments distal to the fibre portion illustrated in Fig. 1. $\times 200$.

FIG. 3 Adaxonal Schwann cell invaginations (s) compartmenting abnormal axoplasm, and particle-filled mitochondrial remnants (m) in a longitudinally sectioned myelinated fibre from a tibial nerve above the ankle. $\times 11\,250$. FIG. 4 Enlarged axon (a) filled with neurofilaments in a cross-sectioned unmyelinated fibre from a plantar nerve. Note excess folds at basal laminae (arrow). $\times 15\,750$. FIG. 5 Enlarged, vacuolated, and degenerating axon in a cross-sectioned unmyelinated fibre from a plantar nerve. $\times 14\,750$.

remyelinated internodes (Fig. 2). In some fibres, isolated internodes displayed a progressive distal enlargement terminating in a bulbous axonal swelling of giant proportions and, in others, some proximal portions of the nerve fibre appeared to be more abnormal than in distal regions. Fibres composed of a chain of ovoids were also found.

Sections of affected nerves showed that the axonal swellings were composed of masses of neurofilaments, and segregated groups of mitochondria and neurotubules. Proximal to these giant axonal swellings, axons of myelinated fibres contained particle-filled mitochondrial remnants and adaxonal Schwann cell invaginations which compartmented abnormal axoplasmic organelles (Fig. 3) (Spencer and Thomas, 1974). These two pathological features were also seen distally where the fibres were also shrunken, contorted, and frequently contained a dense, filamentous axoplasm. In these regions, some myelinated fibres had degenerated and others had been replaced by clusters of regenerating axon sprouts.

Degenerative changes were also seen in unmyelinated fibres. Many displayed a distorted contour associated with layers of irregularly arranged basal lamina, some contained one swollen axon filled with neurofilaments, and in others, one or a few axons were vacuolated and variably enlarged (Figs 4 and 5). The majority of motor nerve terminals of leg and foot muscles appeared normal but a few were swollen and contained a large number of neurofilaments. Mild perivascular oedema was present in the proximal affected areas of nerves. Distally, where nerve fibre degeneration was advanced, there was marked endoneurial and sub-perineurial oedema. Occasional endoneurial mast cells were present in these regions but other haematogenous cells were rare.

DISCUSSION

The present study has shown that 2,5-hexanedione, a major metabolite of the industrial solvent MBK, produces a pattern and distribution of peripheral and central nervous system degeneration which appears similar to that produced by MBK. It is probable, therefore, that

2,5-hexanedione, or one of its metabolites, is responsible for some of the neurotoxic effects of MBK. Although 2,5-hexanedione is commercially available, it seems unlikely that this substance will ever be associated with human neuropathy, since it has a restricted use in industry.

Giant axonal swelling was an early feature of degeneration both of myelinated and unmyelinated nerve fibres in vulnerable areas of the central and peripheral nervous systems. Although paranodal demyelination and remyelination presumably were secondary phenomena in the affected regions of peripheral myelinated nerve fibres, other types of Schwann cell activity were also early features. For example, the adaxonal compartments of Schwann cells invaginated areas of axon with early disease and sequestered abnormal axoplasm, a process which is believed to retard the progressive axonal demise (Spencer and Thomas, 1974). Local Schwann cell movement was also suggested by the presence of surplus folds of basal laminae around distal regions of unmyelinated fibres, a phenomenon the significance of which is obscure. The accumulation of neurofilaments within unmyelinated axons has been described in human giant axonal neuropathy (Carpenter *et al.*, 1974) while the enlargement and vacuolation of these axons is a non-specific feature of unmyelinated nerve fibre degeneration (Thomas, 1974).

The disposition of nervous system change produced by chronic intoxication with 2,5-hexanedione conforms to the sequence of axonal degeneration known as dying-back disease (Cavanagh, 1964). The changes in the affected regions of the peripheral nervous system were predominantly distal but they were also multifocal and involved several internodes. The scattering of disease along the distal regions of vulnerable myelinated fibres may explain why the majority of their motor nerve terminals appeared normal. A multifocal distribution of early nerve fibre degeneration has also been described (Schaumburg *et al.*, 1974) and illustrated (Spencer and Schaumburg, 1974) in the dying-back disease of the nervous system associated with experimental acrylamide intoxication. Additional similarities to acrylamide neuropathy include ongoing nerve fibre regeneration during intoxication and the presence of pro-

nounced pathological changes in nerve fibres prior to discernible clinical symptoms.

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