Journal of Neurocytology **9**, 505–516 (1980)

Ultrastructural studies of the dying-back process. V. Axonal neurofilaments accumulate at sites of 2,5-hexanedione application: evidence for nerve fibre dysfunction in experimental hexacarbon neuropathy

MICHAEL J. POLITIS, RICHARD G. PELLEGRINO and PETER S. SPENCER

Institute of Neurotoxicology, Departments of Neuroscience and Pathology (Neuropathology), Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, New York 10461, U.S.A.

Received 15 August 1979; revised 1 November 1979; accepted 16 November 1979

Summary

This study examines the thesis that 2,5-hexanedione (2,5-HD) produces distal (dying-back) axonopathy by direct toxic action on nerve fibres. Single or repeated application of undiluted or 10% 2,5-HD to exposed rat sciatic nerves caused some fibres to develop focal axonal swellings filled with abnormally large numbers of 10 nm neurofilaments. Such changes occurred within four days of application and were especially prominent in equivalently treated nerve segments obtained from animals orally exposed to 0.5% 2,5-HD before surgery. Nerve fibres along the perimeter of tibial nerve fascicles exposed to 2,5-HD or 2,4-hexanedione (2,4-HD), a compound unable to produce systemic neuropathy, underwent non-specific breakdown and Schwann cell necrosis. Nerve fibres located in the centre of such fascicles only developed an hypertrophied paranuclear Schwann cell cytoplasm and did not proliferate intermediate filaments. Saline, hydrochloric acid and 1,6-hexanediol, a water-soluble hexacarbon also lacking systemic neurotoxic properties, produced no intra-fascicular changes when locally applied to the sciatic nerve. It is concluded (1) that 2,5-HD causes giant axonal swellings by direct toxic action on the nerve fibre, (2) 2,5-HD does not induce a generalized disorder of cytoplasmic intermediate filaments and (3) primary Schwann cell changes produced by locally applied 2,5-HD or 2,4-HD are non-specific and unrelated to the formation of the giant axonal swellings.

Introduction

2,5-Hexanedione (2,5-HD), the gamma diketone metabolite of *n*-hexane and methyl *n*-butyl ketone (M*n*BK) (DiVincenzo *et al.*, 1976), is a primary neurotoxic compound

0300-4864/80/040505-12$03.20/0

useful for studying the etiology of central–peripheral distal (dying back) axonopathy (Spencer & Schaumburg, 1976). Prolonged systemic exposure to 2,5-HD causes long and large central and peripheral nerve fibres distally to develop giant axonal swellings filled with 10 nm neurofilaments (Spencer & Schaumburg, 1977). These focal accumulations of neurofilaments first appear on the proximal sides of nodes of Ranvier and are accompanied by a focal blockade of fast axonal transport at these sites (Griffin *et al.*, 1977; Mendell *et al.*, 1977). The focal swellings also determine the positions from which the distal part of axons subsequently undergoes Wallerian-like degeneration (Spencer & Shaumburg, 1977; Veronesi *et al.*, 1980).

Toxic distal axonopathies of this type are believed to result from injury either to the neuronal soma (Cavanagh, 1964) or to the nerve fibre (Spencer & Schaumburg, 1976; Schoental & Cavanagh, 1977; Spencer *et al.*, 1979). The latter hypothesis is consistent with the observation that 2,5-HD inhibits *in vitro* the activity of glycolytic enzymes in the nerve fibre on which axonal transport is known to depend (Sabri *et al.*, 1979a, b). The present study strongly supports the idea of direct damage to the nerve fibre as the pathological mechanism in hexacarbon distal axonopathy by reproducing the hallmarks of the systemic disease in nerves locally exposed to 2,5 HD.

Materials and methods

Male Sprague–Dawley rats (weighing about 300 g) were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. The neurotoxic compound 2,5-hexanedione and the non-neurotoxic compounds 2,4-hexanedione (2,4-HD) and 1,6-hexanediol (1,6-HDiol) were obtained from Eastman Chemicals, Rochester, New York.

Animals were anaesthetized with sodium barbitone (50 mg/kg of body weight) for the application of test agents to sciatic nerves. The application site in the mid-thigh was prepared by placing a piece of Parafilm between the nerve and the underlying muscle tissue. Vaseline petroleum jelly was then applied to the proximal and distal ends of the exposed portion of the nerve to form a well in which sterile cotton was placed in opposition to the nerve. 250 μl of one of the following solutions was then applied to the cotton: 2,5-HD, undiluted, or as a 10% aqueous solution (pH 4.5), undiluted 2,4-HD, 1,6-HDiol (320 mg/m of water), saline, or an aqueous solution of hydrochloric acid (pH 3.5). Parafilm was placed over the open wound and, after 45 min, the cotton and Vaseline were removed and the area thoroughly irrigated with sterile saline. The sciatic nerve on the opposite side was similarly exposed to saline. For closure of the incision, muscles and skin were separately apposed with 4.0 silk. Hexacarbons were applied to the nerves of some animals daily for periods up to eight days. Some animals received 0.5% 2,5-HD in their drinking water for a period of eight days prior to surgery. All other animals drank water.

For tissue examination 6 h–16 days after the initial surgery, animals were deeply anaesthetized with sodium barbitone containing heparin, and perfused through the heart with 4% paraformaldehyde followed by phosphate-buffered 5% glutaraldehyde (pH 7.4). Sciatic nerves from experimental and control sides were excised, briefly washed or stored for 1–2 days in Sorensen's phosphate buffer, postfixed for 2 h in 2% Dalton's chrome osmium solution at 5° C, dehydrated stepwise in increasing concentrations of ethanol, immersed in propylene oxide and embedded in Epon. After polymerizing the resin blocks, 1 μm sections were cut, stained with toluidine blue and examined with the light microscope. Thin sections of selected areas were stained with uranyl acetate followed by lead citrate, and examined by electron microscopy.

Results

FUNCTIONAL EFFECTS OF HEXACARBON APPLICATION

Marked unilateral hindlimb weakness was seen consistently within 1–2 days after 2,5-HD application to the sciatic nerve. This was most prominent in animals which had also received 2,5-HD orally prior to surgery. Unilateral weakness was also seen at four days in one animal whose sciatic nerve had been exposed to 2,4-HD. All other animals remained functionally normal.

PATHOLOGICAL EFFECTS OF HEXACARBON APPLICATION

Controls

In animals sacrificed at 4, 8, 10 and 16 days postoperatively, nerves exposed to the sham-operation, saline or hydrochloric acid appeared normal except for the presence of some granulation tissue in the epineurium (Fig. 1).

2,4-Hexanedione and 1,6-hexanediol

Light microscope examination of sites of a single 2,4-HD application revealed at four and seven days a nonspecific breakdown of nerve fibres, capillary damage and red blood cell extravasation along the perimeter of the large (tibial, peroneal) nerve fascicles (Fig. 2). The entire cross-section of the smaller (sural) fascicle displayed degenerating fibres. The central part of the larger fascicles appeared morphologically normal except for occasional fibres which, in cross-section, displayed greatly enlarged paranuclear Schwann cell cytoplasm of the type produced by 2,5-HD application (*vide infra*). Ultrastructural examination of the surviving nerve fibres showed that axons contained a normal concentration and distribution of neurofilaments, microtubules and mitochondria. No changes were seen in sciatic nerves treated with 1,6-HDiol.

2,5-Hexanedione

Light microscope examination of nerves at the site of a single 2,5-HD application revealed a gradient of pathological change ranging from complete destruction of nerve fibres along the perimeter of the larger nerve fascicles, to preservation of fibres in the centre of the fascicle (Figs. 3, 4). This pattern probably reflected the effect of a radial concentration gradient of 2,5-HD, the highest levels of toxin existing beneath the perineurium and the lowest in the centre of the fascicle.

By four days, the perimeter of fascicles exposed to 2,5-HD displayed complete, nonspecific breakdown of nerve fibres at the site of toxin application and diffuse Wallerian degeneration distally. Despite the use of perfusion fixation, capillaries in the sub-perineurial zone contained blood cells. These were also encountered in adjacent endoneurial tissue. By 16 days, the necrotic zone contained some small, thinly myelinated (regenerating?) fibres.

Deeper within 2,5-HD-exposed fascicles, between the perimeter of necrotic fibres and the inner zone of preserved fibres, the most prominent structures were swollen myelinated and demyelinated axons (Fig. 3). By light microscopy, many of the swollen

axons had a waxy appearance similar to the giant axonal swellings consistently seen in systemic hexacarbon neuropathy (Spencer & Schaumburg, 1977). Ultrastructural examination of these fibres 4 and 16 days after application of the toxin revealed swollen axons uniformly filled with 10 nm neurofilaments, with microtubules scattered in small clusters or surrounding mitochondria. Myelin sheaths in cross-section were thin or absent (Figs. 5, 6). Demyelinated axons were associated with Schwann cells which displayed an increased number of cytoplasmic intermediate filaments, mitochondria and small vesicles. These changes were not apparent in remyelinating Schwann cells (Fig. 6). By 16 days, the swollen axons frequently

---

Fig. 1. Transverse section of sham-operated control nerve contralateral to site of 2,5-HD application. Nerve fibres appear normal. Granulation tissue (g) is present in the epineurium. × 200. This figure and Figs. 3 and 4 show 1 μm sections, stained with toluidine blue of tissue obtained from rats given 2,5-HD in their drinking water for eight days prior to surgery. The sham-operated side opposite to toxin-exposed nerves shown in all subsequent illustrations appeared similar.

Fig. 2. Large nerve fascicles seven days after exposure to 2,4-HD. Red blood cells appear in the endoneurium (upper right corner). Nonspecific nerve fibre breakdown is widespread in the endoneurium. However, many fibres in the central part of the fascicle (lower left) are preserved. 1 μm section stained with toluidine blue. × 360.

Fig. 3. Tibial nerve fascicle four days after single application of undiluted 2,5-HD. Three morphologically distinct zones are seen: *upper*, adjacent to the perineurium *(p)*, containing nonspecific breakdown of nerve fibres; *central*, displaying swollen myelinated and demyelinated axons; and *lower*, showing axons of normal size and appearance. × 360.

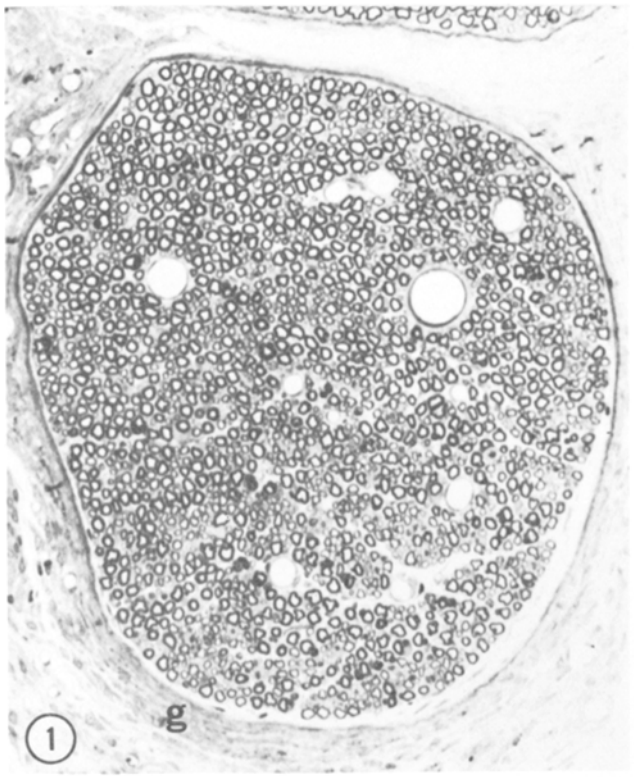
Fig. 4. Tibial nerve fascicle 16 days after a single exposure to undiluted 2,5-HD. Many small, thinly myelinated (regenerating?) axons can be seen. Nerve fibre breakdown is still present. A few large, naked axonal swellings are visible (arrows). × 570.

Fig. 5. Swollen axon in the tibial nerve fascicle 4 days after a single application of undiluted 2,5-HD. The axoplasm is uniformly packed with 10 nm neurofilaments. Small groups of microtubules (arrows) are scattered in the axoplasm, often ajacent to mitochondria *(m)*. The myelin sheath is thin or absent. This figure and Figs. 6 and 7 show thin sections, stained with uranyl acetate and lead citrate, of tissue obtained from rats given 2,5-HD in their drinking water for eight days prior to surgery. × 15 000.

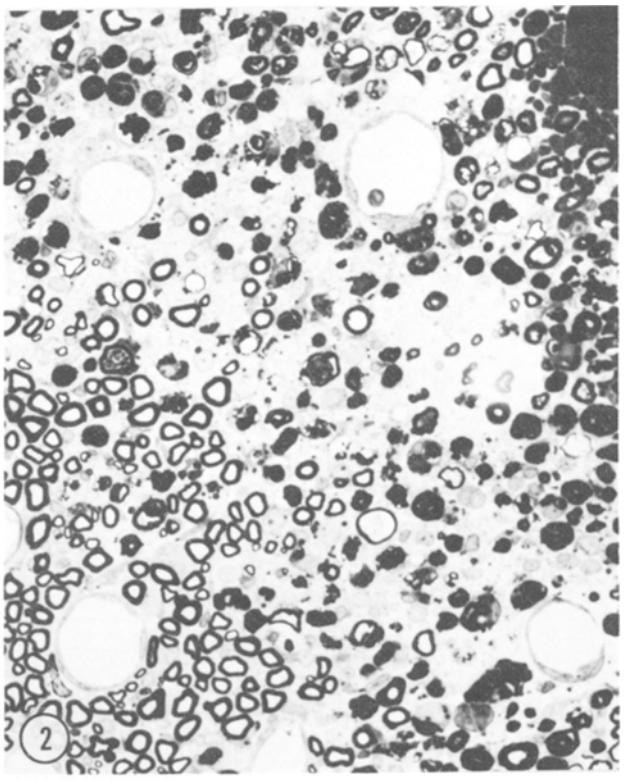
Fig. 6. Swollen, remyelinated axon in a tibial nerve fascicle 16 days after a single application of undiluted 2,5-HD. The axon is packed with 10 nm neurofilaments. × 17 150.

Fig. 7. Swollen axon 16 days after single application of undiluted 2,5-HD. The central region of axoplasm contains neurofilaments, microtubules, mitochondria and smooth endoplasmic reticulum. The surrounding axoplasm is arranged in a circular array. × 19 800.

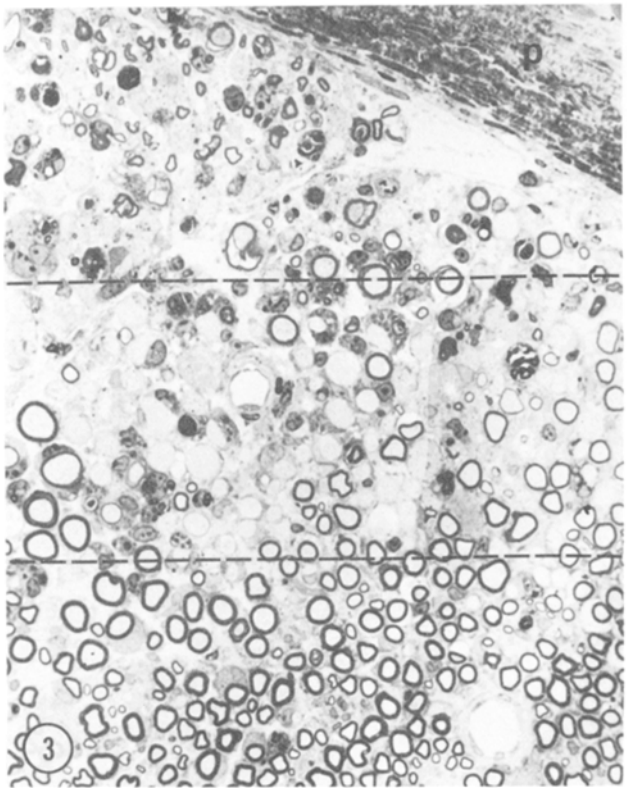
Fig. 8. Fibre from the central region of the tibial nerve fascicle five days after a single application of undiluted 2,5-HD. The Schwann cell nucleus *(n)* is eccentrically located. The enlarged paranuclear cytoplasm contains several vacuoles, numerous mitochondria, a prominent Golgi apparatus and abundant rough endoplasmic reticulum. The axon and myelin associated with this Schwann cell appeared normal. Thin section stained with uranyl acetate and lead citrate. × 14 200. The inset shows a lower power view of this area in which two fibres display marked enlargement of their paranuclear Schwann cell (*) cytoplasm and eccentric placement of nuclei. 1 μm section stained with toluidine blue. × 730.



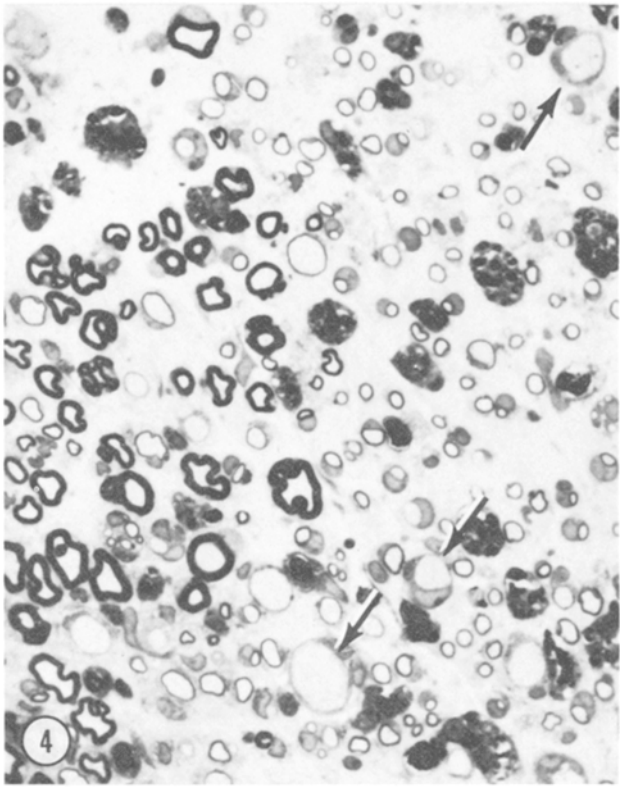
1



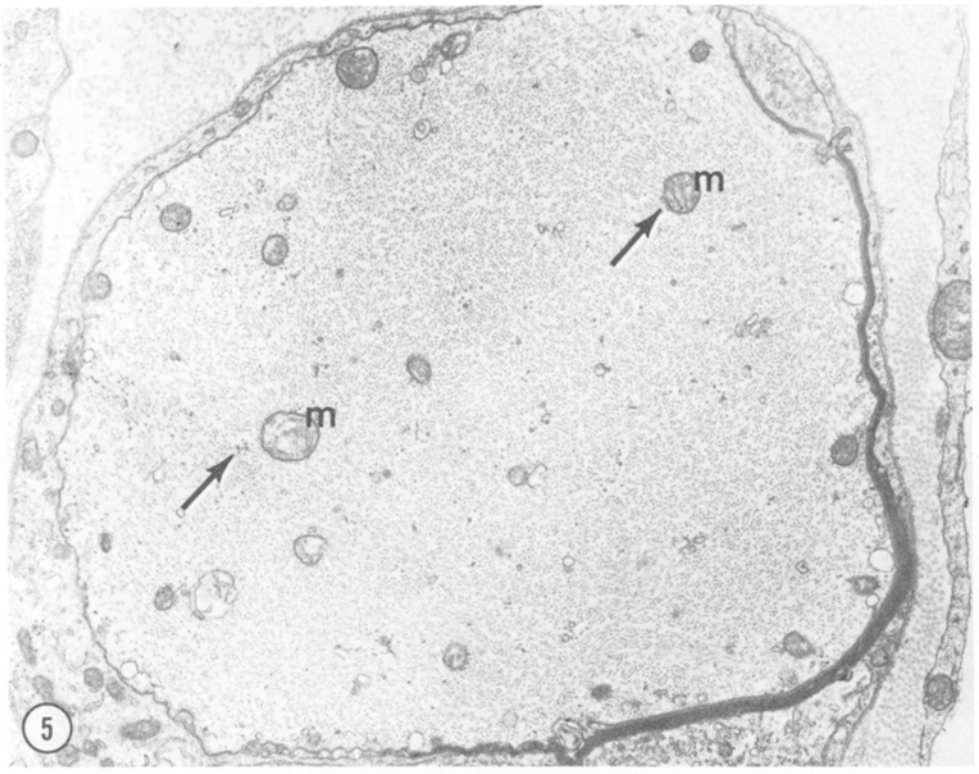
2

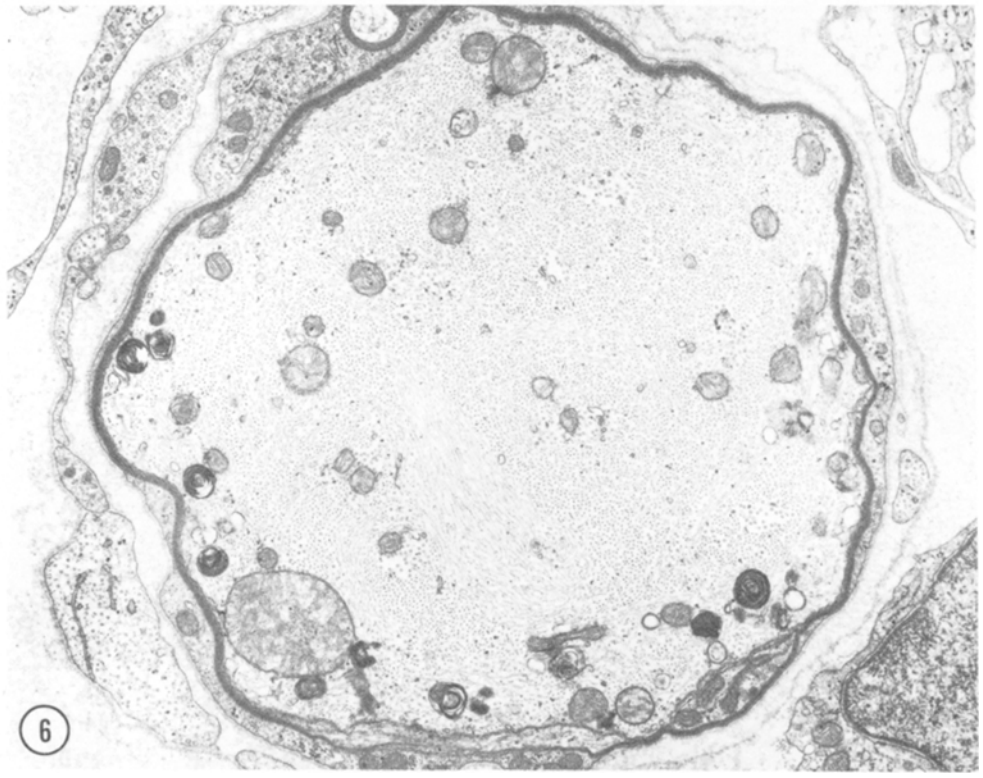


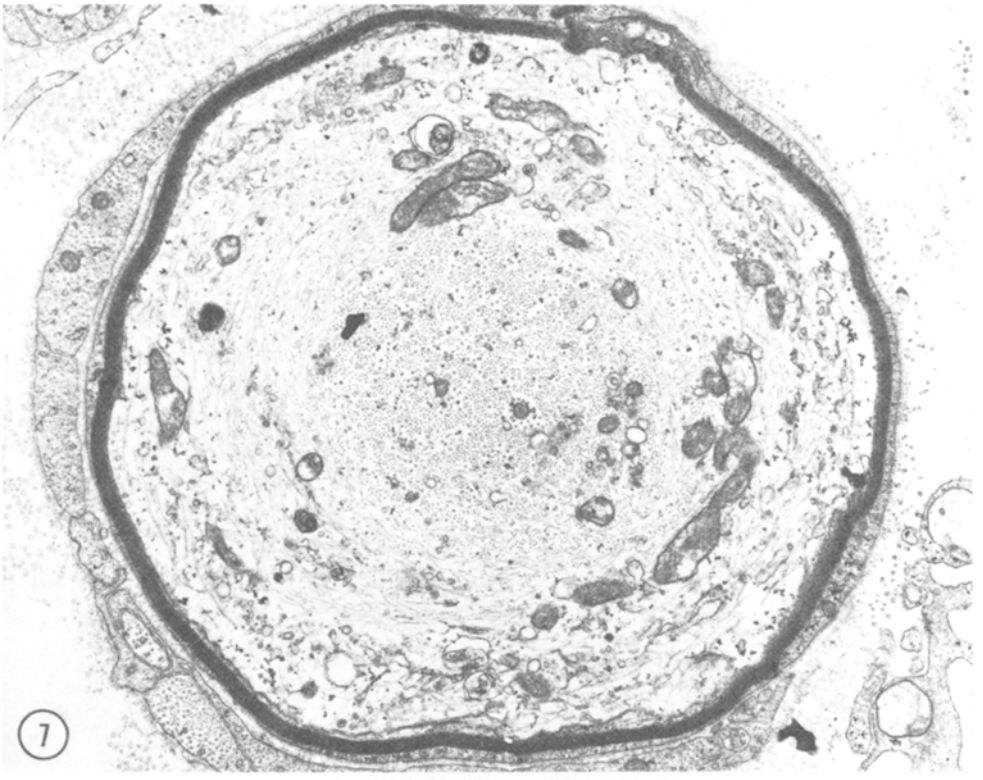
3



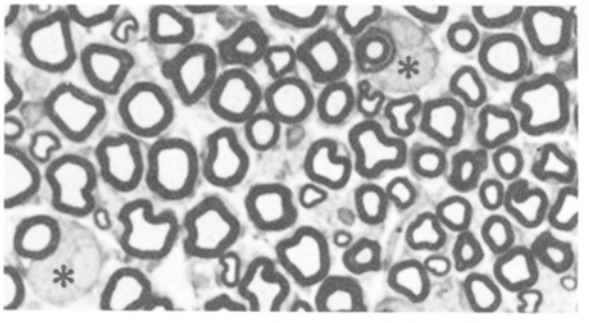
4


m
m
5


6



7



8

displayed a central core of longitudinally-aligned axoplasmic components surrounded by 10 nm neurofilaments, microtubules and mitochondria arranged in a circular array (Fig. 7). Comparable structures have been reported in systemic hexacarbon neuropathy (Mendell *et al.*, 1977; Spencer & Schaumburg, 1977) but their temporal evolution has not previously been clarified. Many swollen and unswollen fibres in this zone had thin myelin sheaths and were surrounded by supernumerary Schwann cells, features indicative of remyelination.

Swollen, neurofilament-filled axons were especially prominent in 2,5-HD-exposed nerves of animals that had received oral 2,5-HD prior to surgery. By contrast, saline or acid-exposed nerves of these systemically 2,5-HD-intoxicated animals, as well as those of unintoxicated rats, appeared normal.

At all time-points, the central part of nerve fascicles exposed to 2,5-HD contained fibres with normal-appearing axons and myelin sheaths. However, in cross-section, many fibres contained an enlarged paranuclear Schwann cell cytoplasm and an eccentrically placed nucleus (Fig. 8, inset). The enlarged cytoplasm contained several vacuoles, numerous mitochondria, a prominent Golgi apparatus and abundant rough endoplasmic reticulum (Fig. 8). This type of change was especially obvious in animals that had received repeated applications of 10% 2,5-HD for five or eight days. However, no clear relationship could be established between duration of exposure and Schwann cell changes.

The incidence of axonal swellings was increased by giving animals drinking water containing 2,5-HD for eight days prior to surgery. However, no qualitative differences were seen between nerves from animals which had and had not been so treated.

Discussion

The present study demonstrates that focal axonal swellings filled with 10 nm neurofilaments, the specific hallmarks of hexacarbon and some other polyneuropathies (*vide infra*), develop in peripheral nerves at sites of 2,5-HD application. This observation provides direct evidence in support of the idea, proposed earlier in this series of papers (Spencer & Schaumburg, 1977), that axonal neurotoxins induce pathological changes in nerve fibres by direct toxic action. Similar claims have been made for two other axonal neurotoxins, namely di-isoflurophosphate (Glazer *et al.*, 1978) and disulphiram (Zuccarello & Anzil, 1979). In view of these observations, it seems very likely that axonal neurotoxins precipitate dying-back nerve fibre disease by their ability to damage nerve fibres directly, rather than by causing dysfunction of neuronal somata.

2,5-Hexanedione, acrylamide and carbon disulfide produce similar types of neurofilamentous distal axonopathies when systemically administered to experimental animals (Spencer *et al.*, 1980). Their common ability to inhibit glycolytic enzymes in nerve tissue incubated *in vitro* has been invoked as evidence of direct toxic action on nerve fibres (Sabri & Spencer, 1980). It has been proposed (Spencer *et al.*, 1979) that

such enzyme inhibition occurs throughout the nerve fibre during systemic intoxication, thereby causing axonal demand for enzyme replacement from the neuronal soma to increase substantially. The soma may manage to resupply only the proximal axon, causing the concentration of enzymes in the distal axon to dwindle. Functions such as axonal transport, which depend on the maintenance of glycolysis for energy support (Sabri & Ochs, 1972), may begin to fail and pathological changes develop. Distally, axons accumulated slowly-transported 10 nm neurofilaments above energy-demanding nodes of Ranvier, the paranodal myelin sheath slips back from the swelling axon and, eventually, the length of axon beneath the swelling undergoes Wallerian-like degeneration (Veronesi *et al.*, 1980).

This hypothesis provided the impetus to compare the effects of 2,5-HD application to peripheral nerves in the presence and absence of systemic, 2,5-HD intoxication. It was reasoned that if systemic administration of 2,5-HD inhibited glycolytic enzymes along the nerve fibre, then the additional local application of 2,5-HD to the sciatic nerve would cause swellings to develope more rapidly than in equivalent regions of systemically unintoxicated animals. The absence of nerve fibre swellings in the sham-operated nerve of the systemically 2,5-HD-intoxicated animals, coupled with the strikingly more prominent swellings in the locally 2,5-HD-exposed nerves of these animals compared to similar regions of systemically unintoxicated animals, provides indirect evidence in support of this rationale. Since the early accumulation of neurofilaments produced by 2,5-HD likely results from a focal blockade of slow axonal transport of these organelles (Griffin & Price, 1980), this new model of 2,5-HD mononeuropathy may prove useful for dissecting the mechanisms involved in the control of axonal transport systems.

Mendell and colleagues (1977) also examined the effects of a neurotoxic hexacarbon (M*n*BK) when applied locally to peripheral nerves. In their experiments, rat sciatic nerves exposed to a sponge soaked in M*n*BK subsequently displayed circumferential, nonspecific nerve fibre breakdown. This was also found in the present study at the perimeter of nerves locally exposed to 2,5-HD, 2,4-HD, but not to the water-soluble compound 1,6-hexanediol. Since 2,4-HD and 1,6-HDiol fail to produce neuropathological changes following repeated systemic administration to rats (Spencer *et al.*, 1978), it is evident that the hexacarbon solvents M*n*BK, 2,5-HD and 2,4-HD, when directly applied in high concentrations to peripheral nerves, exert damaging effects on nerve fibres that are unrelated to the specific ability of 2,5-HD to induce the formation of neurofilamentous axonal swellings. This nonspecific effect of the solvents is to be distinguished from the *specific* pattern of axonal swellings induced only by 2,5-HD *(vide infra).*

Schwann cells maintaining myelinated fibres displayed similar reactions in fascicles exposed to 2,5-HD or 2,4-HD. Necrosis was found in the perimeter of exposed nerves where the concentration of the solvent presumably was highest. Centrally in exposed fascicles, the paranuclear cytoplasm of Schwann cells maintaining myelin was hypertrophied. Mitochondria, endoplasmic reticulum and Golgi apparatus were all

increased suggesting that the cell was unusually metabolically active. Schwann cells exposed to 2,5-HD failed to proliferate cytoplasmic intermediate filaments, except in relation to demyelination. These observations give no support to the suggestion of Prineas and colleagues (1976) that neurotoxic hexacarbons may induce a generalized cellular proliferation of intermediate filaments similar to that reported in the childhood disorder of giant axonal neuropathy (Asbury *et al.*, 1972; Prineas *et al.*, 1976). Some of the Schwann cell changes found in the present study are not too dissimilar from those reported by Powell and colleagues (1978) in murine 2,5-HD polyneuropathy. These authors found Schwann cells with increased numbers of intermediate filaments and abundant smooth endoplasmic reticulum. They suggested that such changes might not be reactive to axonal disease but, instead, might represent one of two primary nerve fibre responses to chronic 2,5-HD intoxication, one involving the axon, and the other the Schwann cell. In support of their suggestion, they reported finding Schwann cell changes concurrently in proximal and distal regions of affected tibial nerves. This was interpreted to indicate a generalized Schwann cell change in response to 2,5-HD intoxication. However, such a conclusion is premature because in systemic hexacarbon polyneuropathy in the rat, nerve fibre changes ascend the tibial nerve from the ankle and calf in two independent waves, such that axonal swellings appear in the proximal portion of the tibial nerve before they appear in the posterior tibial nerve above the ankle (Spencer & Schaumburg, 1977). Thus, the question addressed by Powell and colleagues (1978) can only be studied satisfactorily by examining the proximal and distal regions of an *unbranched* nerve or, preferably, those regions of individually isolated nerve fibres. In the present system of local toxin application, Schwann cell hypertrophy has been demonstrated, but this phenomenon was encountered in nerves locally exposed to 2,5-HD *or* to 2,4-HD, a compound which does not produce polyneuropathy after systemic intoxication (Spencer *et al.*, 1978). We have, therefore, been unable to demonstrate in this study a 2,5-HD-specific Schwann cell abnormality occurring independent of axonal change, but the possibility of a primary Schwann cell response to 2,5-HD cannot be excluded. It seems certain, however, that the functional deficit of neuropathy is related to changes in the axon which result in fibre degeneration, but whether 2,5-HD induces axonal swellings by acting on the axon and/or the Schwann cell primarily remains to be resolved.

Acknowledgements

We are grateful to Monica Bischoff and Richard Conte for technical assistance and Elaine Garafola for preparing the manuscript. This work was supported by NSF grant PFR-78-1270 and USPHS grants OH 00535 (CDC), NS 13106, ES 02168, NS 03356 (NIH). M.J.P. is the recipient of a post-doctoral fellowship from a NRSA Training Grant, NS 07098. RGP is supported by NIH Training Grant 5T 32 GM 7288 from NIGMS.

References

ASBURY, A. K., GALE, M. K., COX, C. S., BARINGER, J. R. & BERG, B. O. (1972) Giant axonal neuropathy. A unique case with segmental neurofilamentous masses. *Archives of Neurology* **20**, 237–47.

CAVANAGH, J. B. (1964) The significance of the 'dying-back' process in experimental and human neurological disease. *International Review of Experimental Pathology* **3**, 319–67.

DiVINCENZO, G. D., KAPLAN C. J. & DEDINAS, J. (1976) Characterization of the metabolites of methyl *n*-butyl ketone, methyl iso-butyl ketone and methyl ethyl ketone. *Toxicology and Applied Pharmacology* **36**, 511–22.

GLAZER, E. J., BAKER, T. & RIKER W. F. (1978) The neuropathology of DFP at cat soleus neuromuscular junction. *Journal of Neurocytology* **7**, 741–58.

GRIFFIN, J. W. & PRICE, D. L. (1980) Proximal axonopathies induced by toxic chemicals. In *Experimental and Clinical Neurotoxicology* (edited by SPENCER, P. S. and SCHAUMBURG, H. H.). Baltimore: Williams and Wilkins.

GRIFFIN, J. W., PRICE, D. L. & SPENCER, P. S. (1977) Fast axonal transport through giant axonal swellings in hexacarbon neuropathy. *Journal of Neuropathology and Experimental Neurology* **36**, 603 (abstract).

MENDELL, J. R., SAHENK, Z., SAIDA, K., WEISS, H. S., SAVAGE, R. & COURI, D. (1977) Alterations of fast axoplasmic transport in experimental methyl *n*-butyl ketone neuropathy. *Brain Research* **133**, 107–18.

POWELL, H. C., KOCH, T., GARRETT, R. & LAMPERT, P. W. (1978) Schwann cell abnormalities in 2,5-hexanedione neuropathy. *Journal of Neurocytology* **7**, 517–28.

PRINEAS, J. (1969) The pathogenesis of dying-back polyneuropathies. II. An ultrastructural study of experimental acrylamide intoxication in the cat. *Journal of Neuropathology and Experimental Neurology* **28**, 598–621.

PRINEAS, J. W., OUVRIER, R. A., WRIGHT, R. G., WALSH, J. C. & McLEOD, J. G. (1976) Giant axonal neuropathy – a generalized disorder of cytoplasmic microfilament formation. *Journal of Neuropathology and Experimental Neurology* **35**, 458–70.

SABRI, M., EDERLE, K., HOLDSWORTH, C. & SPENCER, P. S. (1979a) Studies on the biochemical basis of distal axonopathies. II. Specific inhibition of fructose-6-phosphate kinase by 2,5-hexanedione and methyl *n*-butyl ketone. *Neurotoxicology* **1**, 285–98.

SABRI, M. I., MOORE, C. L. & SPENCER, P. S. (1979b) The biochemical basis of distal axonopathies. I. Inhibition of glycolysis by neurotoxic hexacarbon compounds. *Journal of Neurochemistry* **32**, 683–9.

SABRI, M. I. & OCHS, S. (1972) Relationship of ATP and creatine phosphate to fast axoplasmic transport in mammalian nerve. *Journal of Neurochemistry* **19**, 2821–8.

SABRI, M. I. & SPENCER, P. S. (1980) Toxic distal axonopathy: Biochemical studies and hypothetical mechanisms. In *Experimental and Clinical Neurotoxicology* (edited by SPENCER, P. S. and SCHAUMBURG, H. H.). Baltimore: Williams and Wilkins.

SCHOENTAL, R. & CAVANAGH, J. B. (1977) Mechanisms involved in the 'dying-back' process – an hypothesis implicating coenzymes. *Neuropathology and Applied Neurobiology* **3**, 143–7.

SPENCER, P. S., BISCHOFF, M. C. & SCHAUMBURG, H. H. (1978) On the specific molecular configuration of neurotoxic aliphatic hexacarbon compounds causing central-peripheral distal axonopathy. *Toxicology and Applied Pharmacology* **44**, 17–28.

SPENCER, P. S., SABRI, M. I. & POLITIS, M. (1980) Methyl *n*-butyl ketone, carbon disulfide and acrylamide: putative mechanisms of neurotoxic damage. In *Progress in Neurotoxicology* (edited by MANZO, L., LERTY, N., YACASSE, Y. and ROCHE, L.). Oxford: Pergamon Press.

SPENCER, P. S., SABRI, M. I., SCHAUMBURG, H. H. & MOORE, C. M. (1979) Does a defect of energy metabolism in the nerve fiber underlie axonal degeneration in polyneuropathies? *Annals of Neurology* **5**, 501 (abstract).

SPENCER, P. S. & SCHAUMBURG, H. H. (1976) Central and peripheral distal axonopathy – The pathology of dying-back polyneuropathies. In *Progess in Neuropathology* (edited by ZIMMERMAN, H.), Vol. 3, pp. 253–295, New York: Grune and Stratton.

SPENCER, P. S. & SCHAUMBURG, H. H. (1977) Ultrastructural studies of the dying-back process. III. The evolution of experimental peripheral giant axonal degeneration. *Journal of Neuropathology and Experimental Neurology* **36**, 276–99.

VERONESI, B., PETERSON, E. R. & SPENCER, P. S. (1980) Reproduction and analysis of M*n*BK neuropathy in organotypic tissue culture. In *Experimental and Clinical Neurotoxicology* (edited by SPENCER, P. S. and SCHAUMBURG, H. H.). Baltimore: Williams and Wilkins.

ZUCCARELLO, M. & ANZIL, A. P. (1979) A localized model of experimental neuropathy by topical application of disulfiram. *Experimental Neurology* **64**, 699–703.