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Detection of neurofilament autoantibodies in human serum following chemically induced neurologic disorder: a case report

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Sera obtained from a 5-year-old patient who was exposed to tar and the insecticide, chlorpyrifos (*O*,*O*-diethyl 3,5,6-trichloropyridinyl phosphorothioate), and subsequently developed neurologic deficits consistent with organophosphorus ester-induced delayed neurotoxicity (OPIDN) and from normal healthy controls (members of his family) were assayed by Western blotting for the presence of autoantibodies against the three neurofilament proteins: 200-kDa, outer- or high- molecular-weight (NFH); 160-kDa, middle-molecular-weight (NFM); and 68-kDa, core- or low-molecular-weight (NFL) subunits. High levels of autoantibodies against the NFH and NFM of the neurofilament proteins were detected in the exposed patient's serum. Traces of autoantibodies for NFH were detected in three control sera but those of NFM were detected in two controls only. In summary, the results suggest that chemically induced neurologic disorders such as OPIDN induce an autoantibody response against NFH and NFM components of the neurofilament triplet proteins. *Environmental Epidemiology and Toxicology* (2000) **2,** 37–41.

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Introduction

Studies demonstrated an increased incidence in human sera of autoantibodies formation to intracellular cytoskeletal proteins (Willard and Simon, 1981). Autoantibodies detected in healthy individuals may arise as a normal reaction to tissue turnover, resulting in a release of neuronal proteins capable of stimulating autoreactive B lymphocytes to produce antibodies against these proteins (Fujinami et al., 1983). Alterations in the cytoskeletal structure are pathognomonic features of some neurodegenerative disorders such Alzheimer's disease (Selkoe and Shelanski, 1977), Creutzfeldt–Jacob disease (Sotelo et al., 1980) and some types of hypertrophic cardiopathy (Kurki and Virtanen, 1984). Likewise, changes in cytoskeletal proteins are characteristic of neurodegenerative disorders induced by chemicals such as organophosphorus esters (Jenson et al., 1992; Abou-Donia, 1993), *n*-hexane and metabolites (Lapadula et al., 1988), acrylamide (Reagan et al., 1994), glycidamide (Reagan et al., 1995) and carbon disulfide (Wilmarth et al., 1993).

While most organophosphorus esters exert their toxic action by inhibiting acetylcholinesterase (AChE), a few produce a condition known as organophosphorus ester-induced delayed neurotoxicity (OPIDN; Smith et al., 1930; Abou-Donia, 1981). OPIDN is a neurodegenerative disorder of increasing importance in our population globally exposed to organophosphorus esters. OPIDN is a distal axonopathy of the central and peripheral nervous systems, characterized by a delay period of 6–14 days before onset of ataxia and paralysis accompanied by Wallerian-type degeneration of the axon and myelin. A pathognomonic feature of OPIDN is early ultrastructural alteration of microtubules and neurofilaments that undergo condensation and aggregation followed by their dissolution (Bischoff, 1967, 1970; Prineas, 1969). The etiology of OPIDN is still largely unknown, although there is a considerable body of evidence implicating an aberrant hyperphosphorylation of cytoskeletal proteins such as microtubules, neurofilament triplet proteins, tau proteins, and microtubule-associated proteins (MAP-2), paralleled to increased protein kinases such as activity of calcium/calmodulin kinase II (CaM Kinase II; Abou-Donia, 1993; Abou-Donia and Lapadula, 1990; Jenson et al., 1992; Gupta and Abou-Donia, 1998).

Tri-*ortho*-cresyl phosphate (TOCP), an industrial chemical, first produced OPIDN in humans and later in sensitive species (Smith et al., 1930; Abou-Donia, 1981). The insecticide, chlorpyrifos (*O*,*O*-diethyl 3,5,6-trichloropyridinyl phosphorothioate), produced OPIDN in a 42-year-old man who drank 300 mg chlorpyrifos/kg body weight subsequent to development of acute cholinergic toxicity (Lotti et al., 1986). A 3-year-old boy, who was found playing near an open spilled bottle of Dursban

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(chlorpyrifos), rapidly developed acute cholinergic signs followed by paralysis of the vocal cords after 11 days (Aiuto et al., 1993). Following exposure to Dursban, eight persons developed peripheral neuropathy; five of whom exhibited memory loss and cognitive slowing (Kaplan et al., 1993). In experimental studies, OPIDN was produced following a single oral dose of 60–90 mg/kg ($2-3\times LD_{50}$) in hens (Capodicasa et al., 1991) and 300 mg/kg in cats (Fikes et al., 1992). Recent studies have shown that the threshold dose of chlorpyrifos to produce OPIDN was reduced following concurrent exposure to the non-delayed neurotoxicant, propetamphos, 1-methylethyl(*E*)-3-[[(ethylamino)methoxyphosphinothioy]oxy]-2-butenoate(Abou-Donia and Wilmarth, 1995; Abou-Donia et al., 1998).

This study was carried out to examine sera from a young patient who was exposed to tar and the organophosphorus insecticide, chlorpyrifos, and developed neurologic deficits for the presence of autoantibodies against the subunits of neurofilament triplet proteins. Comparative studies were performed with sera from healthy controls from his family.

Materials and methods

Patient

The 12-month-old patient was exposed to organophosphorus compounds under two circumstances. He played in dirt where roofing tar was shallowly buried. The tar was used to caulk walls to prevent water leaks. The product, as labeled, contained tar (petroleum distillates) and encapsulated asphalt. Tar is known to contain mixtures of phosphoric acid esters (Budavari, 1989) and the neurotoxicants pyridine, phenol, cresol, and xylenol (Perov, 1972; Pinski and Bose, 1988). Around the time of the first episode, a second exposure to the organophosphorus insecticide, chlorpyrifos, took place. This insecticide had been applied on several occasions inside the home, permeating the rugs and upholstery where the child crawled.

Prior to these exposures, there was no history of symptoms of acute organophosphorus insecticide poisoning. The patient's development was normal. He had walked at 8.5 months of age and had normal vocabulary at 1 year of age. The parents date the onset of symptoms from the time of exposure. At first, the onset of cholinergic signs and symptoms was subtle and gradual. He had several episodes of diarrhea without fever in the next 2 months. He also displayed cold symptoms and rashes.

By 14 months of age, 2 months after exposure, the family thought he was unsteady on his feet, and his speech was regressing. He had become irritable. A pediatrician examined him at 16 months of age and his neurologic state was normal. At 17 months of age, 5 months after his first exposure, his inability to walk without falling was recognized as abnormal and the search for potential causes began. By 18 months of age, he could not walk but his deep tendon reflexes were still normal, and he had no clonus. His inability to walk was thought to be due to weakness, but ataxia could not be ruled out. He had no nystagmus and other cerebellar signs were difficult to assess. By 19 months of age, he had developed increased deep tendon reflexes, clonus, and extensor planter reflexes. Within a month, 8 months after exposure, spasticity in the extremities was well-developed. His neck muscles continued to be weak and head control was poor.

Fourteen months after exposure, when the patient was 26 months old, there was some improvement in his speech and his irritability diminished. Other neurological abnormalities persisted. At 7 years of age, 5 years after exposure, his neurological condition was unchanged; his intellect was lower than his family and special school placement was necessary.

Determination of Neurofilament Autoantibodies

The titer of antineurofilament antibody in the sera was determined by a quantitative Western blot assay. Increasing amounts of sera were incubated with nitrocellulose strips containing identical amounts of neurofilament triplet proteins. Conditions were established in which the amount of binding antibodies in sera to neurofilament proteins was proportional to the amount of sera used. Sera used were obtained 4 years after exposure when the patient was 5 years of age, and from his 6- and 9-year-old brothers, 32-year-old father, and 34-year-old mother. The sera were tested for the presence of autoantibodies against the neurofilament triplet proteins: NFH (200 kDa), NFM (160 kDa), and NFL (68 kDa). Neurofilament proteins were prepared fresh from bovine spinal cord by the method of Chiu and Norton (1982). A 10-μg sample of neurofilament proteins was electrophoretically separated on 10-well 7.5% polyacrylamide gels, with 4% staking gels. After electrophoresis, the gels were electrophoretically transferred to nitrocellulose by the method of Towbin et al. (1979) overnight. After transfer, the nitrocellulose blots were air-dried and cut into individual lanes with one lane stained with Coomassie Brilliant Blue R-250 to visualize protein bands. These lanes were then individually probed in duplicate using the immunoblotting described by Wilmarth et al. (1993), using the 100-μl sera as the source of antibodies. The lanes were autoradiographed for 24 h at -70°C with XAR type film, and the resulting autoradiograph scanned with laser densitometer (LKB2202 Ultrasound) for quantification.

Materials

Commercially available materials were purchased from the following sources: [^{125}I] protein A (361.62 Ci/mmol) from Dupont NEN Research Products (Boston, MA), electrophoresis-grade reagents from BioRad Laboratories (Richmond, CA), TEMED and Trisbase from Boehringer

Mannheim (Indianapolis, IN), nitrocellulose (0.2-μm pore size) from Schleicher and Schnell (Knee, NH), and X-OMAT RP-1(8×10 in.) film from Eastman Kodak Company (Rochester, NY).

Results

Laboratory and radiologic examinations have ruled out space-occupying lesions in the head and spinal column, or diseases of mitochondria, fatty acid amino acid metabolism. Skin biopsy found no evidence of storage diseases. A nerve biopsy and electroencephalogram were normal. Fat levels of chlorinated pesticides were normal.

Table 1. Autoantibody binding against neurofilament proteins[a].

Subject	NFH		NFM	
	Densitometer unit	(%)	Densitometer unit	(%)
Patient	129.22	100.00	137.07	100.00
6-year-old brother	0.0	0.0	0.0	0.0
9-year-old brother	4.73	3.66	4.13	3.01
Father	4.25	3.29	0.0	0.0
Mother	6.87	5.32	37.46	27.33

[a]Sera from the 5-year-old patient and normal control were assayed by Western blotting for the presence of autoantibodies against the neurofilament triplet protein: NFH, NFM, and NFL as described under Materials and methods section.

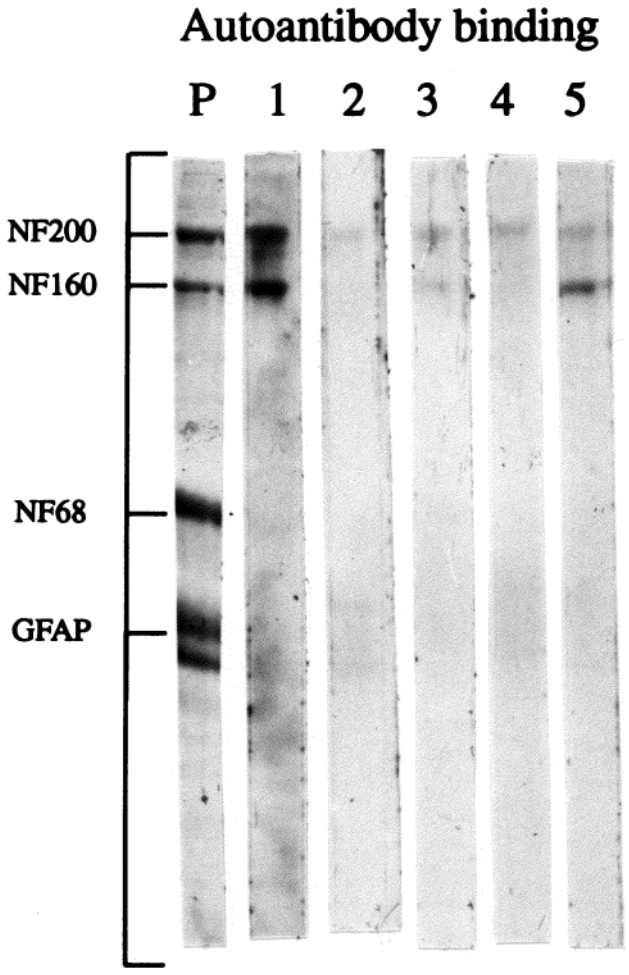

Figure 1. Immunoblots of bovine spinal cord neurofilament-enriched preparation reacted with sera from a patient exposed to tar and the organophosphorus insecticide, chlorpyrifos, who developed neurologic deficits. The designations are: P, protein standards; NFH, 200-; NFM, 160-; and NFL; 68 kDa; (1) 5-year-old male patient who was exposed when he was 1 year old; (2) 6-year-old brother; (3) 9-year-old brother; (4) 32-year-old father; and (5) 34-year-old mother. Antibodies for glial fibrillary acidic protein (GFAP), an astroglial protein, were absent from both the patient and the controls, demonstrating the specificity for detection of autoantibodies against neurofilament proteins in chemically induced neurological disorders.

Sera from the patient and three members of his family, who did not have any complaints, possessed antineurofilaments against NFH with the patient showing greatest binding (Figure 1, Table 1). The relative amount of autoantibody binding to NFH compared to that of the patient was in decreasing order: mother (5.32%) > older brother (3.66%) > father (3.299%) > younger brother (0%). Autoantibodies against NFM were detected only in the sera of the patient, his older brother, and mother. The relative amount of autoantibody binding to NFM was in descending order: the patient (100) > mother (27.33%) > older brother (3.01%) > younger brother (0%). No autoantibodies against NFL were detected in any of the sera tested.

Discussion

We report that a 12-month-old child developed OPIDN following exposure to tar and chlorpyrifos, in agreement with previous studies that chlorpyrifos is capable of producing this neurodegenerative disorder in humans and in experimental animals. Although the young of sensitive species do not develop OPIDN following a single exposure to a delayed neurotoxic organophosphorus compound, they are sensitive to multiple doses (Johnson and Barnes, 1970). In the present reports, the young patient was exposed to chlorpyrifos dermally or via inhalation for several weeks after its application at home, while exposure to the tar was dermal. The exposure may have lasted for several days because of the inability to remove the tar completely from the hair and the scalp. Earlier studies have demonstrated that organophosphorus compounds have more access to the nervous system through skin penetration than gastrointestinal tract (Abou-Donia and Graham, 1978, 1979; Abou-Donia et al., 1983), making skin exposure a more effective route for the development of OPIDN. Also, recent reports have indicated that combined exposure to organophosphorus compounds enhances the development of OPIDN

(Abou-Donia and Wilmarth, 1995; Abou-Donia et al., 1998).

The patient's clinical condition is consistent with OPIDN resulting from exposure to the organophosphorus ester, chlorpyrifos, that was potentiated by tar. This conclusion is supported by the following.

(1) The history of exposure to the two compounds is documented.

(2) The time course of the patient's symptoms is in agreement with OPIDN. Initial symptoms are related to inhibition of AChE enzyme by chlorpyrifos; later symptoms followed the three-phase course of neurologic deficits of OPIDN (Abou-Donia and Lapadula, 1990).

(a) The progressive phase, which develops about 2 months after initial exposure, is characterized by leg weakness, diagnosed as peripheral neuropathy and compatible with lower motor neuron dysfunction. Within 6 months of exposure, upper motor neuron signs developed, resulting from persistent and long-lasting involvement of the central nervous system; the brain and spinal cord, characterized by spasticity. Other central nervous system deficits result in mental changes that included irritability and the cessation of normal development of speech.

(b) Stationary phase followed, during which neurologic abnormalities were static.

(c) Improvement phase ensued with time, as the mental changes have lessened, although the patient was left with a mild degree of mental retardation. During this phase, there was an increased ability to use the hands and arms and the exterior movement of the feet and toes.

The patient's flaccid paralysis improvement resulted from a regeneration of the peripheral nervous system. His continued display of spasticity and inability to fully use his legs and hands were consequences of upper motor neuron deficits resulting from long-lasting damage to the central nervous system, which does not regenerate. Spasticity of the severity displayed by this patient is observed in patients with OPIDN (Jedrzejowska et al., 1980; Cisk et al., 1986).

In this study, we used electrophoretic screening of neurofilament autoantibodies with purified bovine neurofilament proteins as targets. This antigenic preparation contained the three neurofilament proteins: NFH (200 kDa), NFM (160 kDa), and NFL (70 kDa). By immunoblotting technique, it was found that the patient's antibodies recognized NFH and NFM proteins. Other members of the family showed much less content of NFH and only two members contained small amounts of antibodies against NFM. Autoantibodies against cytoskeleton proteins may be formed through the reaction of normal tissue turnover (Fujinami et al., 1983). In healthy individuals, the presence of autoantibodies against cytoskeleton proteins is age-dependent; they increase with age. In the present study, the autoantibodies were detected in the mother's serum than any of the other control.

None of the sera tested in this study exhibited autoantibodies against the NFL protein. This protein forms the internal core of the neurofilament, whereas the NFH and NFM proteins form the outer layers (Linder et al., 1979; Liem and Hutchinson, 1982; Stefansson et al., 1985). Thus, the high and intermediate filament proteins are readily exposed to the autoreactive B lymphocytes, resulting in the formation of autoantibodies. Neurofilament antibodies have been formed in sera of patients with neurodegenerative diseases such as spongiform encephalohepatitis, kuru, and Creutzfeld–Jacob disease (Bahmanyar et al., 1983; Toh et al., 1985) and Parkinson's disease (Elizan et al., 1983).

Conclusion

This report suggests that neuronal damage in an OPIDN patient induced an autoantibody response against the NFH and NFM components of the neurofilament triplet protein. The presence of these autoantibodies may provide a useful marker for diagnosis of chemically induced neurologic disorders such as OPIDN and may help in the development of appropriate treatment.

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References

Abou-Donia M.B. Organophosphorus ester-induced delayed neurotoxicity. *Annu. Rev. Pharmacol. Toxicol.* 1981: 21: 511–548.

Abou-Donia M.B. Involvement of cytoskeletal proteins in the mechanisms of organophosphorus ester-induced delayed neurotoxicity. *Clin. Exp. Pharmacol. Physiol.* 1993: 22: 358–359.

Abou-Donia M.B., and Graham D.G. Delayed neurotoxicity of *O*-ethyl *O*-4-nitrophenyl phenylphosphothioate: subchronic (90 days) oral administration in hens. *Toxicol. Appl. Pharmacol.* 1978: 45: 685–700.

Abou-Donia M.B., and Graham D.G. Delayed neurotoxicity of *O*-ethyl *O*-4-nitrophenyl phenylphosphorothioate: toxic effects of a single oral dose on the nervous system of hens. *Toxicol. Appl. Pharmacol.* 1979: 48: 57–60.

Abou-Donia M.B., and Lapadula D.M. Mechanisms of organophosphorus ester-induced delayed neurotoxicity: type I and type II. *Annu. Rev. Pharmacol. Toxicol.* 1990: 30: 405–440.

Abou-Donia M.B., and Wilmarth K.R. The joint action of chlorpyrifos and safrotin on the development of delayed neurotoxicity in the hen. *Toxicologist* 1995: 15: 205.

Abou-Donia M.B., Graham D.G., Makkawy H.A., and Abdo K.M. Effect of subchronic dermal application of *O*-ethyl *O*-4-nitrophenyl phenylphosphorothiate on producing delayed neurotoxicity in hens. *Neurotoxicology* 1983: 4: 247–260.

Abou-Donia M.B., Abdel-Rahman A.A., Abou-Donia S.M., and Jensen K.F. Dose–response study of propetamphos/chlorpyrifos delayed neurotoxicity in the hen. *Toxicol. Sci.* 1998: 42: 343.

Aiuto L.A., Pavlakis S.G., and Boxer R.A. Life-threatening organophosphorus-induced delayed polyneuropathy in a child after accidental chlorypyrifos ingestion. *J. Pediatr.* 1993: 122: 658–660.

Bahmanyar S., Moreau-Dubois M.C., Brown P., Cathala F., and Gajdusek D.C. Serum antibodies of neurofilament antigens in patients with neurological and other diseases and in healthy controls. *J. Neuroimmunol.* 1983: 5: 191–196.

Bischoff A. The ultrastructure of the tri-*ortho*-cresyl phosphate poisoning studies on myelin and axonal alterations in the sciatic nerve. *Acta Neuropathol.* 1967: 9: 158–174.

Bischoff A. Ultrastructure of the tri-*ortho*-cresyl phosphate poisoning: II. Studies on spinal alterations. *Acta Neuropathol.* 1970: 15: 142–155.

Budavari S. Creosote, Coal Tar. *The Merck Index*, 11th edn., 1989, p. 403.

Capodicasa E., Scapellato M.L., Moretto A., Caroldi S., and Lotti M. Chlorypyrifos-induced delayed polyneuropathy. *Arch. Toxicol.* 1991: 65: 150–155.

Chiu F.C., and Norton W.T. Bulk preparation of CNS cytoskeleton and the separation of individual neurofilament proteins by gel filtration dye binding characteristics and amino acid compositions. *J. Neurochem.* 1982: 39: 1252–1260.

Cisk V., Motika D., and Marosi G.Y. Delayed neuropathy after trichorfon intoxication. *J. Neurol. Neurosurg. Psychiatry* 1986: 49: 222.

Elizan T.S., Casals J., and Yahr M.D. Antineurofilament antibodies in postence phalitic and idiopathic Parkinson's disease. *J. Neurol. Sci.* 1983: 59: 341–347.

Fikes J.D., Zachary J.F., Parker A.J., and Beasley V.R. Clinical, biochemical, electrophysiologic, and histologic assessment of chlorpyrifos-induced delayed neuropathy in the cat. *Neurotoxicology* 1992: 13: 663–678.

Fujinami R.S., Oldstone M.B.A., Wroblewska Z., Frankle M.E., and Koprowski H. Molecular mimicry in virus infection: cross-reactions of measles virus phosphoprotein or herpes simplex virus protein with human intermediate filaments. *Proc. Natl. Acad. Sci. U.S.A.* 1983: 80: 2346–2350.

Gupta R.P., and Abou-Donia M.B. Tau proteins-enhanced Ca^{2+}/calmodulin-dependent phosphorylation by brain supernatant of diisopropyl phosphorofluoridate (DFP)-treated hens: tau mutant indicates phoshorylation of more amino acids in tau by CaM kinase II. *Brain Res.* 1998: 813: 32–43.

Jedrzejowska H., Rowinska-Marcinska K., and Hoppe B. Neuropathy due to phytosol (Agritox): report of a case. *Acta Neuropathol. (Berlin)* 1980: 49: 1163–1168.

Jenson K.F., Haykal-Coats N., Lapadula D.M., and Abou-Donia M.B. Anomalous phosphorylated neurofilament aggregates in central and peripheral axons of hens treated with tri-*ortho*-cresyl phosphate (TOCP). *J. Neurosci. Res.* 1992: 33: 455–460.

Johnson M.K., and Barnes J.M. Age and sensitivity of chicks to the delayed neurotoxic effects on some organophosphorus compounds. *Biochem. Pharmacol.* 1970: 19: 3045–3047.

Kaplan J.G., Kessler J., Rosenberg N., and Schaumburg H.H. Sensory neuropathy associates with Dursban (chlorpyrifos) exposure. *Neurology* 1993: 43: 2193–2196.

Kurki P., and Virtanen I. The detection of human antibodies against cytoskeletal components. *J. Immunol. Methods* 1984: 67: 209–223.

Lapadula D.M., Suwita E., and Abou-Donia M.B. Evidence for multiple mechanisms responsible for 2,5-hexanedione-induced neuropathy. *Brain Res.* 1988: 123–131.

Liem R.K.H., and Hutchinson S.B. Purification of individual components of the neurofilament triplet: filament assembly from the 70,000-Dalton sub-unit. *Biochemistry* 1982: 21: 3221–3226.

Linder E., Kurki P., and Anderson L.C. Autoantibody to "intermediate filaments" in infectious mononucleosis. *Clin. Immunol. Immunopathol.* 1979: 14: 411–417.

Lotti M., Moretto A., Zoppellari R., Rizzuto N., and Barusco G. Inhibition of lymphatic neuropathy target esterase predicts the development of organophosphate-induced delayed polyneuropathy. *Arch. Toxicol.* 1986: 59: 176–179.

Perov O.V. Changes in antioxidative activity of cells and functions of the nucleus by the action of sub-toxic doses of phenol–pyridine mixtures from coal tar. *Bull. Exp. Biol. Med.* 1972: 73: 267–270.

Pinski C., and Bose R. Pyridine and other coal constituents as free radical-generating environmental neurotoxicants. *Mol. Cell Biochem.* 1988: 84: 217–222.

Prineas J. The pathogenesis of dying-back polyneuropathies. An ultrastructural study of experimental TOCP intoxication in the cat. *J. Neuropathol. Exp. Neurol.* 1969: 28: 571–597.

Reagan K.E., Wilmarth K.R., Friedman M., and Abou-Donia M.B. Acrylamide increases *in vitro* calcium and calmodulin-dependent kinase-mediated phosphorylation of rat brain and spinal cord neurofilament proteins. *Neurochem. Int.* 1994: 25: 133–143.

Reagan K.E., Wilmarth K.R., Friedman M., and Abou-Donia M.B. *In vitro* calcium and calmodulin-dependent kinase-mediated phosphorylation of rat brain and spinal cord neurofilament proteins is increased by glycidamide administration. *Brain Res* 1995: 671: 12–20.

Selkoe D.J., and Shelanski M.L. In: (Nandy K., and Sherwin I., Eds.), *The Aging Brain and Senile Dementia*, Plenum Press, New York, 1977, p. 247.

Smith M.I., Elvove E., and Frazier W.H. The pharmacological action of certain phenol esters with special reference to the etiology of so-called ginger paralysis. *Public Health Rep. US*: 1930: 2509–2524.

Sotelo J., Gibbs Jr. C.J., and Gajdusek D.C. Autoantibodies against axonal neurofilaments in patients with kuru and Creutzfeldt–Jacob disease. *Science* 1980: 210: 190–193.

Stefansson K., Marton L.S., Dieperink M.E., Molnar G.K., Schlaepfer W.W., and Helgason C.M. Circulatory autoantibodies to the 200,000-Dalton protein of neurofilaments in the serum of healthy individuals. *Science* 1985: 228: 1117–1119.

Toh B.H., Gibbs Jr. C.J., Gajdusek D.C., Goudsmit J., and Dahl D. The 200- and 150-kDa neurofilament proteins react with IgG autoantibodies from patients with Kuru, Creutzfeldt–Jacob disease, and other neurologic diseases. *Proc. Natl. Acad. Sci. U.S.A.* 1985: 82: 3485–3489.

Towbin H., Staehelin T., and Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U.S.A.* 1979: 76: 4350–4354.

Willard M., and Simon C. Antibody detection of neurofilaments. *J. Cell Biol.* 1981: 89: 198–205.

Wilmarth K.R., Viana M.G., and Abou-Donia M.B. Carbon disulfide inhalation increases Ca^{2+}/calmodulin-dependent kinase phosphorylation of cytoskeletal proteins in the rat central nervous system. *Brain Res.* 1993: 628: 293–300.