762 THE PROMOTION OF ORGANOPHOSPHATE POLYNEUROPATHY IN HEN CENTRAL AND PERIPHERAL NERVOUS SYSTEM. M Peraica, A Moretto, F Fioroni, and M Lotti, Istituto di Medicina del Lavoro, Universita' di Padova, Italy

Diisopropylfluorophosphate (DFP) causes delayed polyneuropathy in hens by inhibiting >70% of axonal neuropathy target esterase (NTE). Phenylmethanesulfonyl fluoride (PMSF) protects from DFP polyneuropathy, when given before DFP at doses inhibiting 30-50% of NTE. Doses of PMSF, inhibiting ≥40% of NTE, promote polyneuropathy in hens pretreated with subneuropathic doses of DFP. Selective effects (protection, initiation, promotion) in central or peripheral nervous system have been achieved in hens by means of systemic and/or intra-arterial (sciatic artery) injections of NTE inhibitors. Clinical and biochemical end-points have been used. Systemic initiation by DFP (0.3 mg/kg s.c.) was followed by promotion in the sciatic nerve obtained by injection of PMSF (7 mg/kg) in the sciatic artery. When given alone, DFP caused 60-65% NTE inhibition throughout the nervous system and no polyneuropathy, whereas PMSF caused similar inhibition in the ipsilateral nerve only (about 30% elsewhere). Hens treated in this way developed a monolateral flaccid neuropathy. It was concluded that promotion occurs in peripheral axons. Selective initiation of polyneuropathy in spinal cord only was obtained by DFP (0.3 mg/kg s.c.) given after protection of both sciatic nerves with PMSF (0.55 mg/kg) in both sciatic arteries. This PMSF pre-treatment caused 35-40% NTE inhibition in both sciatic nerves and 20% inhibition in spinal cord. Hens treated in this way and then challenged with PMSF (120 mg/kg s.c.) developed bilateral spastic ataxia. It was concluded that promotion also occurs in spinal cord axons.

763 CARBON DISULFIDE MEDIATED PROTEIN CROSS-LINKING AS A MECHANISM FOR DISULFIRAM INDUCED NEUROPATHY. W M Valentine, F Rimmele, V Amarnath, K Amarnath, and D G Graham. Duke University Medical Center, Durham, NC.

A distal sensorimotor neuropathy has been reported in susceptible patients receiving disulfiram for alcohol aversion therapy. The clinical signs and neurofilamentous swellings present in the myelinated axons of affected patients resemble those produced in humans and animals by chronic low level exposure to CS_2. This suggests that CS_2 liberated from disulfiram may be the ultimate toxicant in disulfiram induced neuropathy. We have investigated the ability of *N,N*-diethyl dithiocarbamate (DEDTC), the anticipated intermediate between disulfiram and CS_2, to produce CS_2 and result in covalent cross-linking of proteins under physiological conditions. Incubation of DEDTC with model proteins produced high molecular weight species as determined by denaturing polyacrylamide gel electrophoresis. Covalent cross-linking proceeded more rapidly for DEDTC than for an equimolar concentration of CS_2. Using ^{13}C NMR, cross-linking was determined to progress through dithiocarbamate formation on protein amino groups with subsequent generation of *bis*(thiocarbamoyl) disulfide and thiourea cross-linking structures. These results demonstrate the ability of DEDTC to liberate sufficient CS_2 to produce covalent cross-linking under physiological conditions and suggest protein cross-linking may be useful for monitoring patients on disulfiram therapy.

764 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CARBON DISULFIDE (CS_2) INDUCED CYTOSKELETAL ALTERATIONS IN THE RAT CENTRAL NERVOUS SYSTEM. KF Jensen[1], KR Wilmarth[2], JK Olin[2] and MB Abou-Donia[2]. [1]US EPA Research Triangle Park, NC. [2]Duke Univ. Med. Center, Durham, NC.

Male Sprague-Dawley rats were exposed to 200 ppm concentrations of CS_2 via inhalation for 10h/day over 140 consecutive days. Control animals were housed in inhalation chambers and supplied with ambient air for the duration of the study. Animals were perfused 24h following the last exposure, with 4% paraformaldehyde in 0.1 M acetate buffer. Brains and spinal cords were removed and processed for immunohistochemistry. Sections were stained with monoclonal antisera against phosphorylated and nonphosphorylated neurofilaments (SMI-31, SMI-33, Sternberger Monoclonals Inc.) or vimentin (V9, Boehringer Mannheim). Enlarged axons were apparent in the spinal cord and brainstem of treated animals. These abnormal axons were not restricted to regions containing the largest axons indicating that this exposure schedule may alter smaller axons as well as larger ones. In treated animals, neurofilament aggregations could be demonstrated with either SMI-31 or SMI-33. The aggregations appeared to differ in the relative amounts of phosphorylated and nonphosphorylated neurofilaments, suggesting the concomitant presence of various states of axonal degeneration and regeneration. The antisera against vimentin faintly labeled the region around the central canal of the spinal cord in control animals. In contrast, distinctive staining was consistently observed in the ventral columns, the tip of the dorsal horn, and the central canal in treated animals. We are currently investigating the cellular localization of CS_2 induced vimentin-like staining. (Sponsored in part by NIOSH Grant OH00823.)

765 EVIDENCE FOR CROSS LINKING OF NEUROFILAMENT (NF) PROTEINS IN RAT SPINAL CORD FOLLOWING INHALATION EXPOSURE TO CARBON DISULFIDE (CS_2). KR Wilmarth, ME Viana, and MB Abou-Donia. Duke Univ. Med. Center, Durham, NC.

Chronic exposure to CS_2 produces morphological changes in the distal regions of long myelinated fibers characterized by perinodal accumulation of neurofilaments in axonal swellings. CS_2 has also been reported to bind neuronal proteins *in vivo* and covalently crosslink bovine serum albumin *in vitro*. In this study, male Sprague-Dawley rats were exposed to 50, 100, or 200 ppm CS_2 via inhalation 12h/day for 140 consecutive days. 24h following the end of treatment, animals were sacrificed and cytoskeletal proteins were isolated from spinal cords of individual animals. Proteins were resolved by SDS-PAGE and stained with Coomassie Blue, or transferred to nitrocellulose and probed with monoclonal antisera against NF200, 160 and 68kD. We observed a significant decrease in the intensity of protein staining and immunoreactivity of all three NF protein bands as well as decreased immunoreactive breakdown products in animals exposed to 200 ppm CS_2. A dose dependent increase in the appearance of multiple bands migrating between 260 and 400 kD with immunoreactivity against NF 160 and NF68 antibodies was also observed. The relative increase in immunoreactivity against these proteins with increasing levels of exposure was NF160>NF68. These results suggest that CS_2 induced cross linking of NF proteins may be involved in the aggregation of NFs characteristic of this neuropathy. (Sponsored in part by NIOSH Grant OH00832.).

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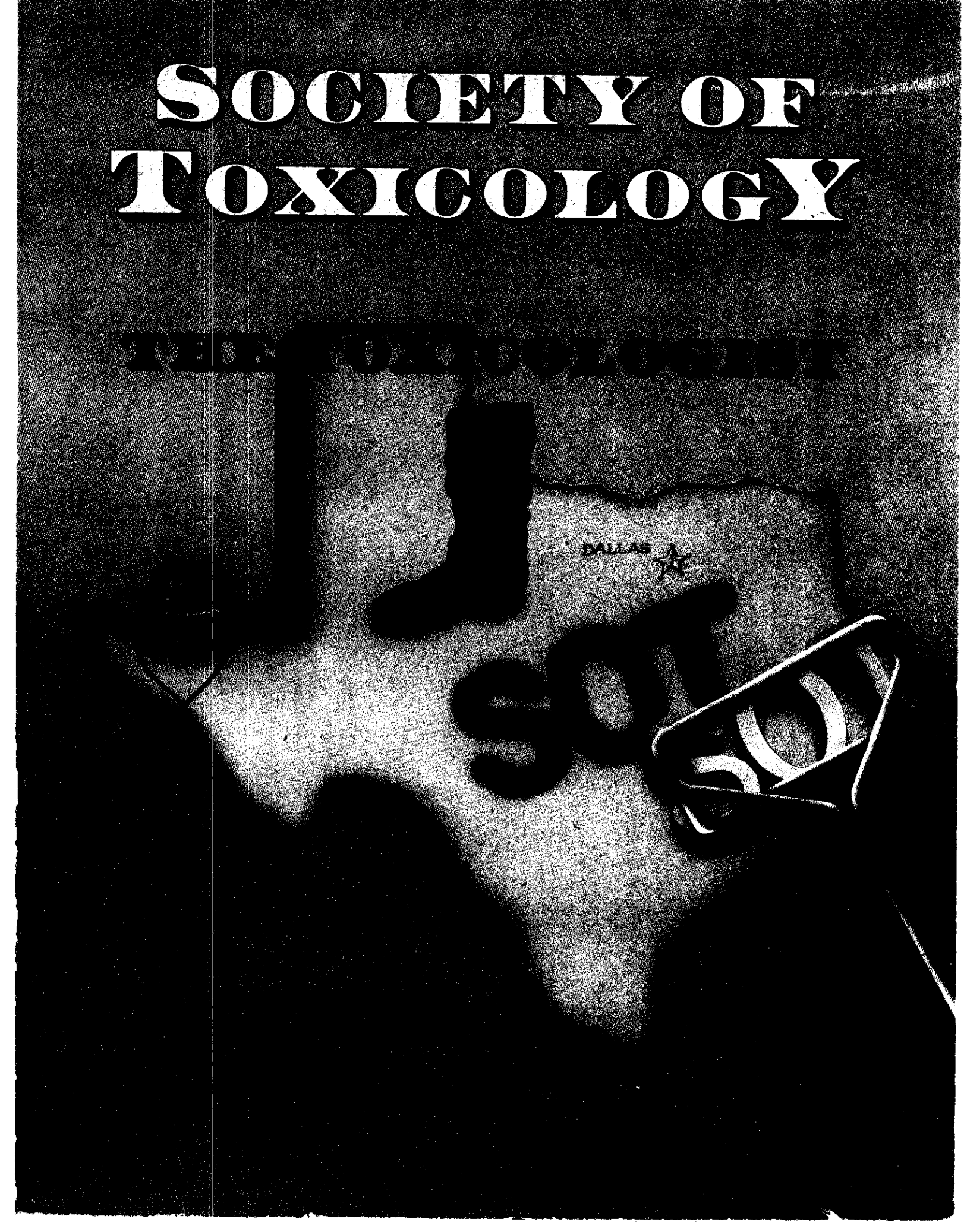

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster/discussion, and poster sessions of the 33rd Annual Meeting of the Society of Toxicology, held at the Loews Anatole Hotel, Dallas, Texas, March 13-17, 1994.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 439.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 467.

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