1762 AN ORGANOPHOSPHATE FROM THE SOLUBLE FRACTION OF MAMMALIAN LIVER.

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We have recently demonstrated that the mammalian paraxonase gene (PON1) is a member of a multi-gene family comprising at least two other genes, PON2 and PON3, which are expressed in several different tissues. The high degree of homology among the deduced amino acid sequences encoded by these genes indicates that the PON1, PON2, and PON3 proteins may share some biological properties. We have partially purified an organophosphatase from the soluble fraction of dog liver. Preliminary results with the partially purified dog enzyme showed that it hydrolyzes several PON1 substrates, such as DFP, soman, and sarin, but, unlike PON1, it does not hydrolyze paraaxon and phenylacetate. The molecular weight of this organophosphatase was estimated to be about 40 kD. These results, including substrate specificity, are similar to those reported by Broomfield et al (1989) for a soluble organophosphatase isolated from rat liver. We have also detected an organophosphatase without paraxonase activity in the soluble fractions of mouse and turkey livers. These results show that, in the species studied, there is at least one other enzyme with the property of hydrolyzing several organophosphates, but which is distinct from PON1. We are continuing our purification studies in order to determine, by sequencing, whether this other organophosphatase is either PON2 or PON3.

1763 NEUROPATHY IN MAN FOLLOWING ISOENOPHS AND PHOSPHIN POISONING.

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A 26-y-old male voluntarily ingested a commercial formulation of the organophosphate pesticides isophenthos (40%) and phoslin (10%); estimated doses were 500 and 125 mg/kg bw and 3 hours after poisoning in plasma levels were 3.5 and 0.1 mg/l, respectively. About 30 min after poisoning, mild cholinergic signs (confusion, vomiting and diaphoresis) were present and the patient was treated with gastric lavage, activated charcoal, 1 mg iv Atropine and 4 g iv 2-PAM. High (>80%) inhibition of plasma cholinesterase and erythrocyte acetylcholinesterase was found 3 hours after poisoning. Treatment with Atropine (7 mg/24 hours) and 2-PAM (8 g/24 hours) lasted 5 days with tapering off over the following 7 days. Cholinergic signs lasted about 1 week and diaphoresis lasted 4 days. The patient was discharged on day 17, 5 days later, complaining of lower limb paresthesia and weakness and within 4 days he was tetraplegic. Electrophysiological studies were indicative of motor (but not sensory) axonal degeneration in both upper and lower limbs. A diagnosis of organophosphate induce delayed neuropathy (OPIDP) was made. About 20 months after poisoning the patient displayed a spastic paraparesis. Movements and strength as well as the electrophysiological data of the upper limbs were approaching normality. Isophenthos but not atropine or 2-PAM is effective in rats at doses well above the LD50. A case of a mild isophenthos phoslin neuropathy associated with a severe cholinergic syndrome has already been reported. However, in our case a discrepancy was observed between the severity of OPIDP and the relatively mild cholinergic syndrome suggesting a possible interfering effect of phoslin.

1764 STANDARDIZING BLOOD CHOLINESTERASE DETERMINATIONS FOR MONITORING PESTICIDE EXPOSURES.

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Cholinesterases (ChE) such as erythrocyte (RBC) acetylcholinesterase (AChE) and plasma cholinesterase (BChE) are used to monitor exposure of organophosphates and organocarboxymides pesticides and to set safe levels for their use. In California, monitoring of blood ChEs is required for pesticide applicators. There are no standards for ChE determinations. This project studies the reliability and interconvertibility of assays based on the Ellman assay which colorimetrically record hydrolysis of acetylthiocholine (AChT) and similar substances. We found that ChE activities using the conditions of the manual Boehringer Mannheim (B/M) kit (the one most frequently used by clinical laboratories in California) were 25-30% lower than those obtained under optimal conditions due mainly to inappropriate ACTh concentration (5.4 mM vs 1 mM and pH 7.2 vs 8.0). To establish factors to convert B/M assay to Ellman assay activities blood was drawn from 9 subjects and diluted with buffer to mimic reduced ChE activity as such expected from pesticide exposure. Total ChE and AChE activities (using glututathione, a selective BChE inhibitor) were determined under optimal conditions for the Ellman assay and those recommended by B/M. Triplicate assays were performed with a 96 well plate reader at 410 nm and 25 °C. Correlation factors obtained were Ellman = 1.39 B/M ± 0.0097 for B/M, and Ellman = 1.34 B/M ± 0.0689 for whole blood ChE (RBC AChE and plasma BChE). (Supported by Cal EPA, NIOSH, US Air Force and NIEHS.)

1765 DO CATECHOLAMINES AND THEIR OXIDATIVE METABOLITES AFFECT SOMAN-INDUCED TOXICITY?

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Soman (GD) is an organophosphonate known to produce cardiac arrhythmias & cardiac failure. Catecholamines (CAs) such as norepinephrine (NE), epinephrine (E), and dopamine (DA) play a key role in the GD cardiac toxicity. There is evidence that increases in CA levels counterbalance the sustained acetylcholine elevation as a result of GD poisoning. The resulting cardiac lesions observed from GD resemble the pathology of a CA lesion. However, metabolites of CAs such as adrenochrome (AC) & adrenolutin (AL) are known to exhibit toxicity that could also account for observed GD toxicity. Using a repeated measures design, monkey serum was analyzed (samples taken at: 5, 15, 30, 45, 60, 120 and 240 min after GD). Animals were pretreated with midazolam (0.0375 mg/kg), IM, 30 min before GD (4xLD50; control serum taken) and IM atropine SO (0.4 mg/kg) & H1 (30 mg/kg) followed by diazepam (150 mg/kg), 15 & 30 sec, after GD. The HPLC separation used a Phase II ODS column & the following mobile phase: 0.13 M monochloroacetic acid, 0.86 mM SOS, 0.67 mM Na EDTA and 2.5% acetonitrile. An electrochemical detector was used for NE, E and DA analysis (+0.80 V) & a UV/visible unit (310, 285 nm) for AC & AL. Preliminary data were consistent with small increases in CAs. The approximately 2000% increase in chromatographic peak within 45 min following GD may support the presence of a CA oxidative metabolite (i.e., AL) which may provide a possible cause for GD toxicity.

1766 INCREASED WHITE BLOOD CELL COUNTS OCCURRED AMONG PEOPLE EXPOSED TO SARIN GAS ON THE TOKYO METRO.

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In the course of investigation aimed to evaluate toxic effects occurred among the victims suffered from nerve gas attack by a religious cult on the Tokyo metro, we happened to analyze that some samples showed high counts of white blood cell in the blood samples (WBC) gathered at the day of the exposure. We intended to get evidence that the increase in WBC depend on the exposure of sarin which is assumed to be a responsible toxicant. Subjects & Methods: Subjects available were 18 persons from two hospitals,