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Environmental Contamination and Toxicology

Effect of Sublethal Levels of Diazinon: Histopathology of Liver

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Diazinon (O, O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothiate) and several other organophosphorus (OP) insecticides exert their toxic effects by inhibiting cholinesterases in many different animals (Davies and Holub 1980; Hussain et al. 1981). Only a few reports are available on the histopathology of pesticides on internal organs and the majority of these studies are mainly restricted to fish tissues (Patel and Chakrabarti 1982; Sastry and Sharma 1981; Anees 1978; Dikshith et al. 1975). This investigation, determined whether low doses of diazinon, a widespread and frequently used insecticide, exerts a histopathological effect in addition to its anticholinesterase action.

MATERIALS AND METHODS

Twenty eight adult male Wistar rats (300-400 gms) were housed in separate cages in a temperature controlled (24°C) room and fed a diet of standard powdered chow for an acclimation period of 30 days. Animals were divided into three groups. Group I, consisting of four rats were sacrificed, untreated at the beginning of the experiment as initial controls. Twelve animals in Group II were fed diazinon (technical grade 87% dissolved in a water-ethanol solution (1:1), twice weekly at a dose of 0.5 mg/kg body weight for twenty eight weeks, by gavage. The exact dosage for each animal was corrected for individual body weight each week by appropriate volume adjustments. Group III, consisting of twelve animals served as controls and received an equivalent amount of the water-ethanol mixture by the same method. All animals were weighed daily. Four control and experimental animals were sacrificed after 7, 14 and 28 weeks respectively. At necropsy, liver tissue was taken and immediately fixed for light and electron microscopy, in 10% paraformaldehyde buffered with S-collidine. In each case, ten to fifteen serial sections from two to three blocks of tissue fixed in Zenker's formalin were cut at 8 microns and stained with hematoxylin and eosin (H & E), and Lillie's azure-eosinate at pH 7.0 (Lillie 1948) for

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detection of cell hypertrophy and margination of cytoplasmic granules in hepatocytes. Frozen sections (8μ) of liver were also stained with Oil Red O and examined microscopically for the presence of "neutral" fat. Lipid accumulation was labelled 'absent' or 'minimal' (0,+), 'moderate' (++), 'severe' (+++), or 'very severe' (++++). All estimates of lipid accumulation were made using a double blind protocol.

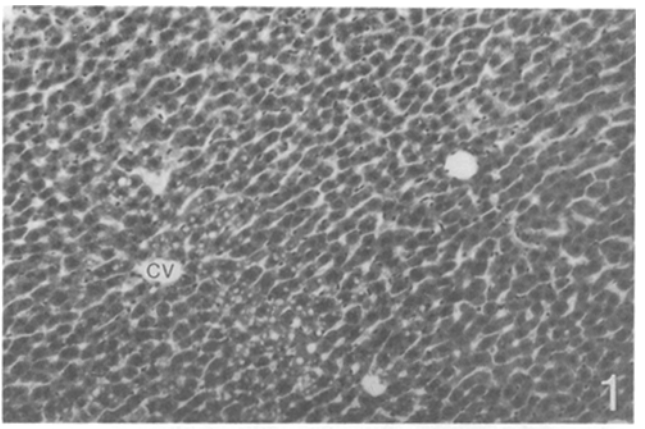
For electron microscopy, tissues were postfixed in osmium tetraoxide and embedded in epoxy resin. Adjacent ultra thin sections, each 40 - 60 μm were stained with uranyl acetate and lead citrate for electron microscopic examination. One micron thick sections were also stained with toluidine blue and examined by the light microscope, in order to evaluate their general morphologic characteristics and to correlate light and electron microscopic findings. Random examination of several liver sections by light microscopy were supplemented by the study of 15-20 serial ultra thin sections of three or four tissue blocks from each biopsy.

RESULTS AND DISCUSSION

No gross disease was observed in animals sacrificed at necropsy. Average daily food intake showed no significant deviations from control values. However, twenty-eight week control animals gained significantly ($p<0.05$) more body weight (602.50 ± 21.08 g) than diazinon treated rats (542.00 + 58.12 g).

The normal lobular architectural pattern of the liver was well preserved in both experimental and control groups (Figure 1). There was, however, evidence of vacuolation or fatty change in the hepatocytes both after 14 and 28 weeks of treatment in experimental animals. Sections of liver from animals treated with diazinon for seven weeks contained slight lipid infiltration in the form of small discrete droplets randomly distributed that were graded (+) compared with the respective control condition (+). After 14 weeks, although there was no evidence of cellular necrosis, there were numerous small and large vacuoles in the perilobular areas of many serial sections of these animals indicative of fatty infiltration (Figure 2) which were graded (++). After twenty-eight weeks, fatty infiltration progressed to include centrilobular areas (Figure 1 and 3) and this was graded (+++) in the experimental groups. Several serial sections showed conglobation of intracellular fat (development of large fat globules) which occasionally displaced the nucleus of the hepatocyte (Figure 3).

Electron microscopic examination of serial sections from 4 blocks randomly selected from whole liver tissue confirmed the light microscopy finding of cellular lipid infiltration (Figure 3). Fat droplets were often observed close to mitochondria. The fine structure of liver in both control and experimental animals is shown in Figures 4 and 5. Abundant rough and smooth endoplasmic reticulum, mitochondria and glycogen were present in cells from



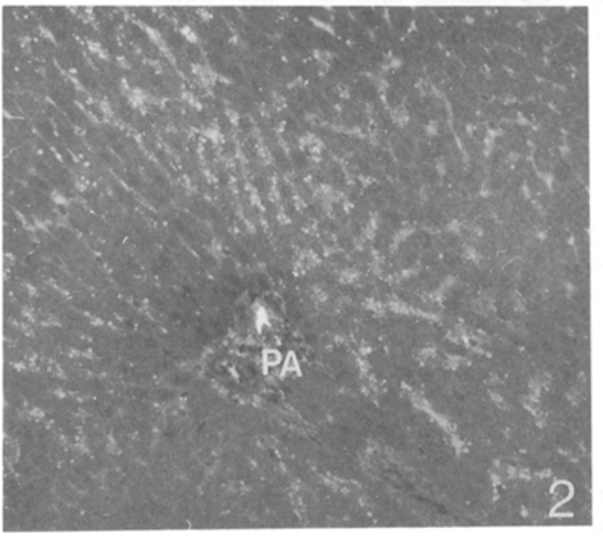


Figure 1. Centrilobular lipid infiltration in a rat exposed to sublethal levels of diazinon for a period of twenty-eight weeks. The lobular architecture of the liver is preserved. CV = central vein. H & E. X 145.

Figure 2. Lipid infiltration present as vacuoles in periportal zone of liver of a rat exposed to sublethal levels of diazinon for a period of fourteen weeks. Note absence of cellular necrosis. One micron thick section stained with toluidine blue. PA = portal canal. X 226.

tissue of both groups. No changes were observed in the nucleus or nucleolus of the hepatocytes from any control or experimental animal.

Histological examination of the liver revealed that animals chronically treated with sublethal doses of diazinon sustain a form of hepatic injury characterized by cellular lipid accumulation. Lipid accumulation is a common hepatic response to

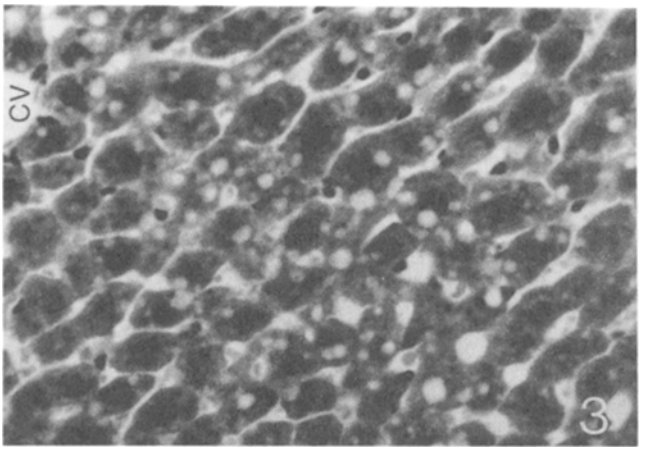


Figure 3. Focus of lipid infiltration in liver. High power of the centrilobular area of Figure 1. CV = central vein. H & E. X 468.

toxic agents such as carbon tetrachloride (Castro et al. 1973); phosphorous (Jacqueson et al. 1977) and chlorinated hydrocarbon insecticides (Ortega et al. 1957).

Various mechanisms may cause this lipid accumulation. They include disturbances of the hepatocellular rough endoplasmic reticulum (Smuckler et al. 1962); increased lipid mobilization from peripheral tissue (Brodie and Maickel 1963) and impaired release of lipoprotein from the liver cell (Recknagel 1967). Some investigators believe that the factors responsible for lipid accumulation in the liver differ from those responsible for hepatic cellular necrosis (Recknagel 1967). Other evidence however, suggests that some toxins significantly increase lipid peroxidation within the liver which ultimately causes necrosis of affected parenchymal cells (Comporte et al. 1965; Recknagel 1967). Since lipid accumulation was prominently observed in the present study, it may be indicative of a much earlier manifestation of liver damage (Recknagel et al. 1958) and slow cell death.

Recently the pathology of various tissues has been studied in fish chronically exposed to sublethal doses of OP insecticides. Fresh-water teleost exposed sublethally to diazinon exhibited cellular necrosis, liver cord disarray and vacuolation in the cytoplasm of hepatocytes (Anees 1978; Sastry and Sharma 1981). The vacuolation observed by these authors might actually have resulted from extensive lipid infiltration, similar to that observed in the present study. Choudhari and Chakrabarti (1984) have also reported that the OP, acephate (orthene) inhibits release of very low density lipoprotein (VLDL) from the liver and have suggested that it is due to a disturbance in the hepatic synthesis of lipoproteins. The present finding of

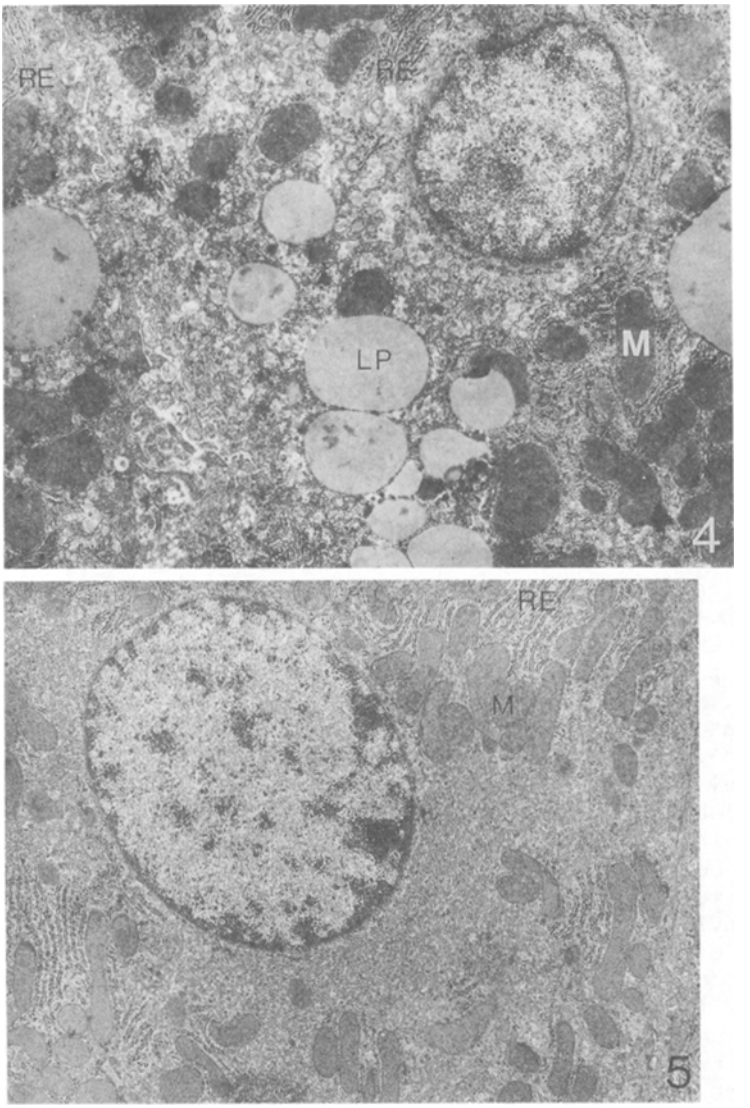


Figure 4. Electron micrograph of liver of a rat fed sublethal levels of diazinon for a period of twenty-eight weeks. LP = lipid, RE = rough endoplasmic reticulum, M = mitochondria. X 15438.

Figure 5. Electron micrograph of liver of a control rat. Note normal morphology of cell. RE = rough endoplasmic reticulum, M = mitochondria. X 8246.

increased lipid accumulation in the liver following prolonged treatment with diazinon however still does not resolve the question of whether impaired lipid metabolism and/or storage is the primary effector.

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