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Cytological Changes in the Fish Liver Following Chronic, Environmental Arsenic Exposure

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In 1973, the Special Studies Section of the Texas Water Quality Board first reported high levels of arsenic in Finfeather and Municipal lakes in Bryan, Texas in Brazos County (CEARLEY, unpublished). One source of the arsenic contamination was an industrial firm manufacturing arsenical dessicants used as cotton defoliants and arsenical pesticides. Seepage of arsenic-contaminated wastes occurred from unreinforced waste treatment ponds into the headwaters of Finfeather Lake, approximately 1 km upstream from Municipal Lake. Levels of arsenic in the water were reported at 240 ppm in the headwaters of Finfeather Lake and at 0.30 ppm in Carters Creek, which was approximately 7 km from the manufacturing firm. These levels exceed the Texas Water Quality Board permissable discharge level of 0.05 ppm and the U. S. Public Health Service recommended drinking water level which is set at 0.01 ppm.

Arsenic levels in sediments at a depth of 5 to 15 cm at 10 locations in Finfeather Lake ranged from 100 to 12,000 ppm and averaged 4700 ppm (CEARLEY, unpublished). Some tissues and the carcasses of seven fish species were observed to have accumulated arsenic to levels exceeding 0.5 ppm (dry weight) which is the Food and Drug Administration maximum permissable limit for arsenic in food.

The liver is the target organ in arsenic poisoning of mammals (HARVEY 1975) and fish (SORENSEN et al. 1979a). Levels of arsenic in the livers of endemic populations of Lepomis cyanellus (green sunfish) and L. macrocharis (bluegills) averaged 2.2 ppm (± 1.8 standard deviation). These levels were four orders of magnitude greater than those levels known to induce histological and ultrastructural changes (SORENSEN 1976a, SORENSEN et al. 1979a) in L. cyanellus following laboratory exposures to one of the least toxic inorganic arsenicals (i.e., sodium arsenate) (SORENSEN 1976b). For this reason green sunfish were sampled from Finfeather and Municipal lakes and livers were processed for optical and electron microscopy to determine whether cytological changes occurred.

MATERIALS AND METHODS

During 1976 and 1977, green sunfish were collected by hook and line from both Finfeather and Municipal lakes in the Navasota River drainage system. In April 1977, fourteen fish were collected

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and transported in water from the collection site, held in an environmental chamber at the same temperature under constant fluorescent lighting, and processed in the Cell Research Institute two days later. Control fish were collected from an area containing no detectable arsenic and were processed in an identical fashion as Municipal Lake fish. Following anesthesia with 0.1 mM benzocaine, lateral and inferior incisions were made in the body wall for exposure and removal of the liver. Two sections of the liver were removed, one for paraffin embedding prior to optical microscopy (OM) and the other for plastic embedding prior to electron microscopy. Tissue designated for paraffin embedding was fixed in Bouin's fixative, washed 24 h, dehydrated in an ethanol and amyl acetate series, and infiltrated with paraffin prior to embedding. Sections (4 μm thick) were stained with hematoxylin and eosin for photomicrography. Tissue blocks were fixed for electron microscopy at room temperature in buffered 3% gluteraldehyde (pH 7.2), washed, and post-fixed in buffered osmium tetroxide. The tissue was block stained overnight in 1% aqueous uranyl acetate, dehydrated in ethanol and propylene oxide, and embedded in Epon 812. Sections were cut with a Porter-Blum MT-1 ultramicrotome. Thick sections (0.5 μm) were stained with 0.1% toluidine blue (TRUMP et al. 1961) and examined with an Olympus Vanox optical microscope. Thin sections (pale gold) were stained with 1% aqueous uranyl acetate and Reynolds lead citrate (REYNOLDS 1962) and examined with a Hitachi HU 11-E electron microscope (operating at 50 kV).

The remainder of the livers from fish collected from Municipal Lake were analyzed by neutron activation to determine the concentration of arsenic in individual livers as previously reported (SORENSEN 1976b). Levels of arsenic ranged from 6 to 64 ppm in these livers (SORENSEN et al., in press). Control fish livers contained no detectable arsenic (SORENSEN 1976a).

RESULTS AND DISCUSSION

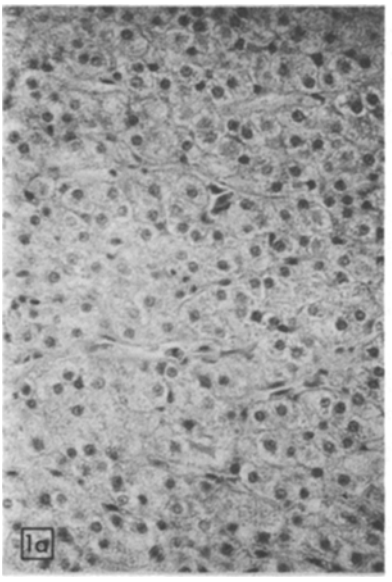
On the April 1977 collection day, the arsenic concentration of Municipal Lake water was 14 ppm; during the four month period prior, levels ranged from 1 to 20 ppm according to atomic absorption analyses for total arsenic which was conducted by the Texas State Health Department during release of water from Finfeather Lake downstream into Municipal Lake (MAYHEW, personal communication).

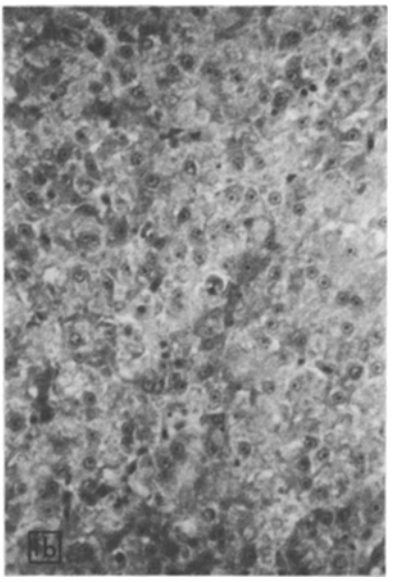
Livers which had accumulated the highest levels of arsenic (i.e., 20 to 64 ppm, on a dry weight basis) showed more extensive histological damage than those containing less arsenic. Abnormally shaped parenchymal hepatocytes were arranged in a disorganized fashion, resulting in an irregular lobular architecture. Many nuclei were displaced from a central location within hepatocytes and contained intensely-stained, central nucleoli which were surrounded by clear areas (Figure 1a and 1b). An average of 39% of nuclei (i.e., 650 μm^2 of liver tissue) contained intensely-stained nucleoli compared with 20% of nuclei from untreated

control fish (SORENSEN et al. 1979a). These nucleoli did not contain birefringent material under polarized light, which suggests the absence of large deposits of a metal or metalloid in paracrystalline arrays within these nucleoli (personal observation). Differential cytoplasmic staining resulted in the presence of light and dark cells especially in proximity to portal veins (Figure 1c); occasional sinusoids were distended and vacuolization of the cytoplasm of hepatocytes was observed (Figure 1d) as previously reported by GILDERHUS (1966).

The most pronounced change observed was submassive necrosis, which was more extensive in livers which accumulated the highest levels of arsenic (Figure 1d). Focal necrosis of hepatocytes has been reported by VON GLAHN et al. (1938) in rabbits administered sodium arsenate, in bluegills treated with sodium arsenite (GILDERHUS 1966), and in humans treated for psoriasis with Fowler's solution (1% potassium arsenite) (LANDER et al. 1975).

Other hepatocytes contained a number of variably pyknotic circular profiles which were sharply delineated from surrounding hepatocytes by the absence of cytoplasmic organelles (Figure 1e and 1f). At the ultrastructural level some of these pyknotic profiles were autophagic vacuoles and lysosome-like dense bodies; others contained lamellar whorls of rough endoplasmic reticulum, electron dense inclusions similar to those produced by laboratory exposures of this species to arsenic (SORENSEN 1976), and numerous vesicles (SORENSEN 1979c) (Figure 2). These membrane-bound structures, designated "necrotic bodies", are known to occupy a greater volume of the hepatocyte in livers containing higher concentrations of arsenic (SORENSEN et al. 1979b). Although necrotic bodies have not been reported to our knowledge in other





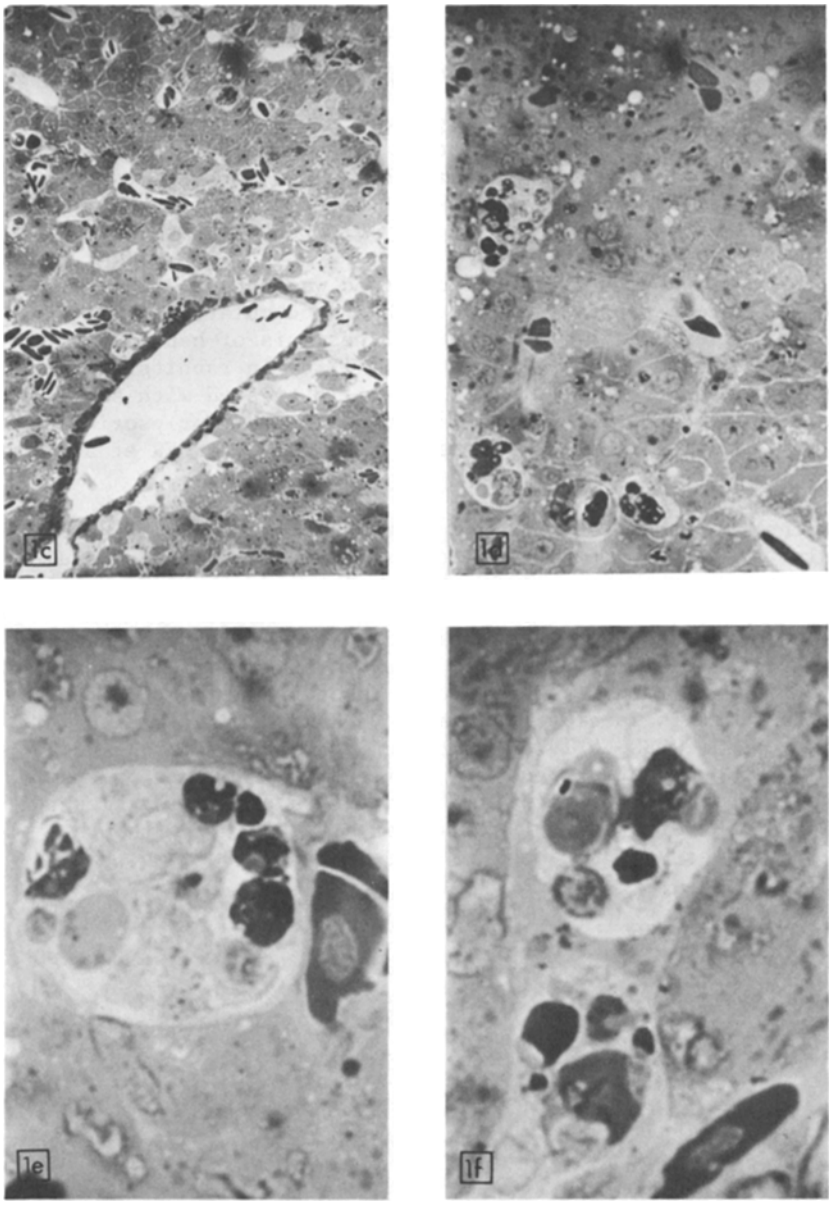


Figure 1. Paraffin-embedded liver from untreated control fish (a) and from sunfish exposed to arsenic in Municipal Lake (b), H & E, 274 X. Epon-embedded and toluidine blue-stained liver from arsenic-exposed fish (c) 259 X, (d) 649 X, and (e) and (f) 1739 X.

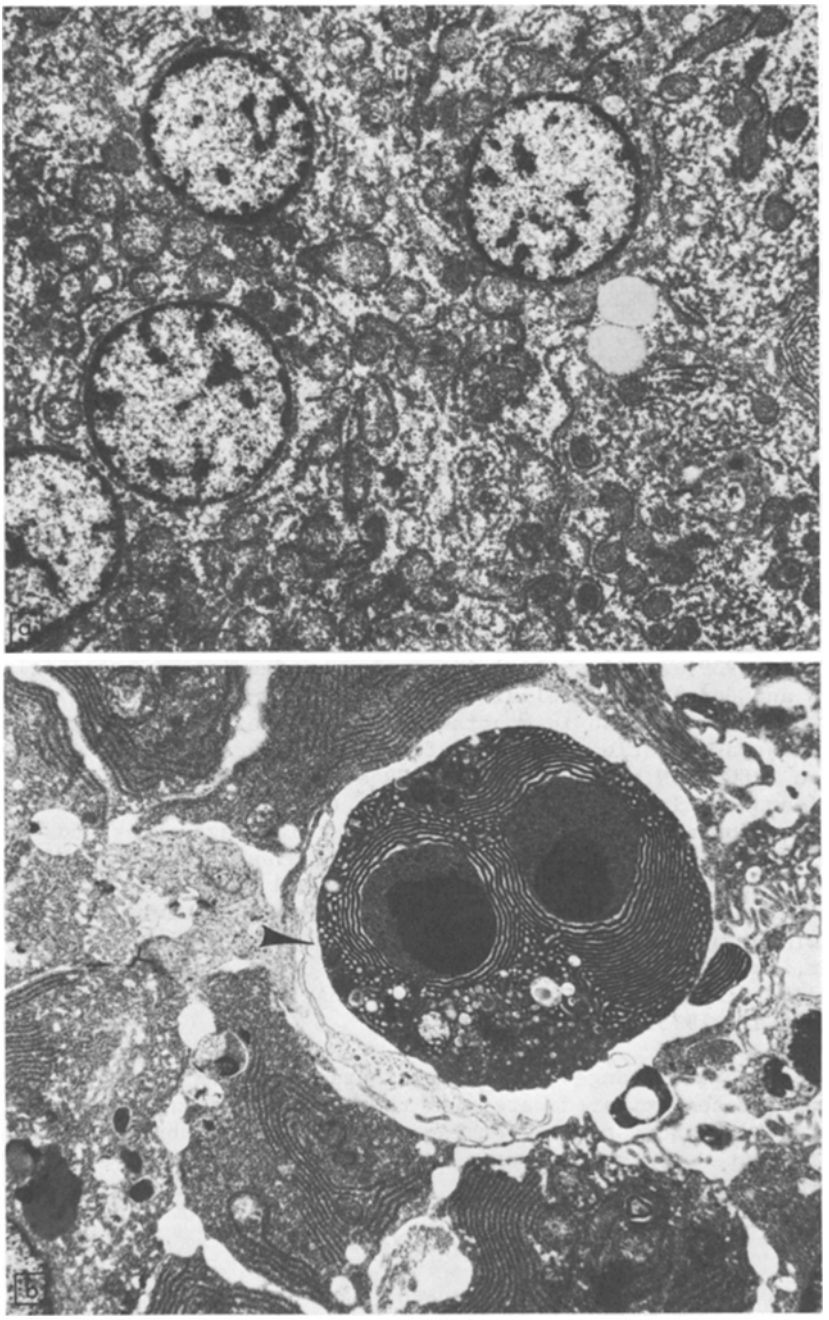


Figure 2. Hepatocytes from a control green sunfish from an area containing no detectable arsenic (a) and from sunfish exposed to arsenic in Municipal Lake (b). A large necrotic body with electron dense deposits, numerous vesicles, and lamellar whorls of RER is indicated (arrow). Uranyl acetate and lead citrate post-stains were used (5453 X).

studies involving arsenic exposure of vertebrates, another form of unicellular degeneration (Councilman or acidophilic body) has been observed to occur in humans exposed to the arsenical gas arsine, AsH_3 (NEUWIRTOVA et al. 1961).

Necrotic bodies were found in various locations -- within sinusoids, in the space of Disse, between hepatocytes, and in hepatocytes. Although the necrotic body has never been observed in the livers of control fish, it appears to be a nonspecific degenerative change (H. POPPER, personal communication). The presence of such bodies within the cytoplasm of other, apparently normal hepatocytes of arsenic-poisoned fish is indicative of the heterophagic capacity of these cells (TRUMP et al. 1973). The presence of necrotic bodies, autophagic vacuoles, dense bodies, light- and darkly-stained parenchyma, and swollen hepatocytes gave livers of these green sunfish a disorganized appearance.

A number of pathological changes occasionally observed following arsenic poisoning were not observed in these fish livers. VON GLAHN et al. (1938) data show bile duct proliferation and bile plugs in the livers of rabbits (but not rats or ferrets) following prolonged feeding of arsenicals. No proliferation of intrahepatic bile ducts or bile duct plugs were found in green sunfish livers, however, as was the case in the BYRON et al. (1967) and FINNER & CALVERY (1939) studies involving prolonged feeding of arsenicals to rats and dogs. Endothelial cell hypertrophy was absent in livers of green sunfish in contrast to that observed in the livers of two psoriasis patients treated for 15 to 17 years with Fowler's solution (REGELSON et al. 1968, LANDER et al. 1975). The hemangioendothelial sarcomas observed in these patients appear to result from more prolonged, chronic arsenical exposures and have not been observed in the two-year exposures of dogs, rabbits, rats, or ferrets (VON GLAHN et al. 1938, BYRON et al. 1967). Fibrosis and cirrhosis was observed by some investigators (FRANKLIN et al. 1950, BUCHANAN 1962, REGELSON et al. 1968); however, none was observed in L. cyanellus or in other vertebrates exposed to arsenic (HUET et al. 1975, ZACHARIAE et al. 1974, LANDER et al. 1975).

The observed changes in livers of green sunfish collected from arsenic-contaminated waters of the Navasota River drainage system are an interesting assemblage of cytological abnormalities which indicate a rather dramatic deviation from normal liver function.

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