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Hemosiderin Granules: Cytotoxic Response to Arsenic Exposure in Channel Catfish

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The deposition of metals and metalloids intracellularly has been reported for lead (BLACKMAN 1936, GOYER et al. 1970, GOYER 1973), gold (NOVIKOFF and HOLTZMAN 1970, GOYER 1973), iron (NOVIKOFF and HOLTZMAN 1970), bismuth (GOYER 1973, PAPPENHEIMER and MAECHLING 1934), uranium (TANNENBAUM 1951), beryllium (GOLDBLATT et al. 1973), mercury (GRITZKA and TRUMP 1968), copper (SCHEUER et al. 1967), and arsenic (SORENSEN 1974, 1976). Although the exact mechanism(s) of metal and metalloid storage is/are not completely understood, such intracellular deposition is/are assumed to provide a means of sequestering extremely toxic metals in a less toxic, stable form, thereby protecting the more vulnerable mitochondrial and endoplasmic reticulum membranes.

Arsenic deposition, as an intranuclear inclusion in the hepatocytes of Lepomis cyanellus (green sunfish), has been verified by numerous techniques including conventional transmission electron microscopy (SORENSEN 1976), high voltage electron microscopy (SORENSEN and SMITH 1980), optical microscopy (SORENSEN et al. 1979a), electron probe X-ray microanalysis (SMITH and SORENSEN 1980), and biochemically (SORENSEN et al. 1979b). The arsenic inclusion, however, has never been reported to our knowledge in any other species, presumably due to morphological similarity between the inclusion and the nucleolus of parenchymal hepatocytes at the optical level. The purpose of this study was, therefore, primarily comparative in nature -- to determine whether a second teleost species (Ictalurus punctatus, channel catfish) shows a similar cytotoxic response following chronic exposure to aqueous solutions of arsenic.

MATERIALS AND METHODS

Adult channel catfish, Ictalurus punctatus, were placed individually in 40 l aquaria containing synthetic lake water solutions and 0 or 15 ppm arsenic as sodium arsenate for approximately six months. Solutions were constantly aerated, filtered (with glass wool only), and changed every two to three weeks throughout the experiment. Solutions were equilibrated thermally (to 20°C) and oxygenated for 1 to 2 days prior to movement of fish to clean solutions; handling stress was minimized as much as possible. Fish were fed Purina catfish chow and exposed to constant fluorescent lighting throughout the experiment.

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At sacrifice fish were anesthetized with MS-222, weighed to 0.1 g and measured for total length. The caudal peduncle was cut and blood collected for a variety of procedures including hematocrit determination, leucocyte differentials, and serum proteins (results will be published elsewhere). Livers were removed and weighed prior to preparation for examination by conventional transmission electron microscopy (CTEM), optical microscopy (OM), scanning electron microscopy (SEM), and electron probe X-ray microanalysis (XRMA).

Liver tissue processed for CTEM and OM was cubed (about 0.5 mm^3) and fixed 2 hr in 3% gluteraldehyde in phosphate buffer (pH 7.3), rinsed in phosphate buffer, and post-fixed in 1% osmium tetroxide in phosphate buffer for 2 hr. Tissue was rinsed twice in water (15 min each), dehydrated in ethanol to 70% and refrigerated in 1% uranyl acetate in 70% ethanol overnight. After completion of the dehydration sequence, tissue was embedded in Spurrs' low viscosity medium, placed in Beem capsules, and polymerized at 60°C overnight. Semithick (1 µm) and thin (pale gold) sections were cut using glass knives on a Sorvall MT-1 ultramicrotome and stained with toluidine blue for OM or uranyl acetate and lead citrate for CTEM, respectively. A Vanox research microscope was used for photomicrotography of semithick sections and a Phillips 200 electron microscope was used for transmission electron micrographs.

Liver tissue processed for XRMA and SEM was rapidly removed and frozen in liquid propane to prevent translocation of elements within the tissue. Blocks of tissue (3 to 4 mm^3) were excised from the liver and stabilized in position on the end of a hollow brass cylinder using finely minced liver as an adhesive. The liver tissue blocks were frozen by immersion in liquid propane cooled in a liquid-nitrogen bath. After an overnight storage in liquid nitrogen, the tissue was transferred to a cryostat maintained at -30°C and 2 µm thick sections were cut with a Minot custom microtome. Sections were placed on carbon-coated nylon which was placed over a 2 mm hole in a 3.5 cm carbon planchett to reduce the generation of X-ray continuum from the supporting planchett. Sections were flattened in position using a formvar film suspended in an aluminum retainer. Sections were freeze-dried under vacuum for 16 hr using a custom made cryosorption apparatus containing molecular sieve beads (type 4A). After the specimen chamber was allowed to warm to room temperature, it was carefully vented to atmospheric pressure with dry nitrogen. Tissue was immediately transferred to a vacuum desiccator for storage. For examination and analysis, the aluminum retainer holding the formvar film was removed from the upper surface of the specimen. Sections were examined in the uncoated condition at 15 kV in a JSM-35 JEOL scanning electron microscope equipped with a Si(Li) X-ray detector. X-ray data was collected, stored, and processed by an NS-800 Tracor Northern pulse height analysis system. The Tracor Northern Super ML multiple least squares fitting routine was used to accomplish peak deconvolution. The program is capable of resolving As L and Mg K peaks at 1.28 and 1.25 keV, respectively. The secondary

image was photographed from the cathode ray tube for morphological examination of areas rastered for analysis of elemental composition. A large number of electron lucent structures were analyzed; dot maps for arsenic, iron, and the continuum were made of these structures to determine elemental distribution within the electron lucent structures. Morphological comparisons were made between scanning electron micrographs and transmission electron micrographs of these structures.

RESULTS AND DISCUSSION

Transmission electron micrographs of liver tissue from untreated control I. punctatus show well-preserved hepatocytes with normal nuclei containing electron-dense nucleoli, numerous mitochondria, rough endoplasmic reticulum (RER), and other ultrastructural features of a typical vertebrate liver cell. Small hemosiderin deposits were also occasionally observed in parenchymal hepatocytes (Figure 1). In contrast, hepatocytes

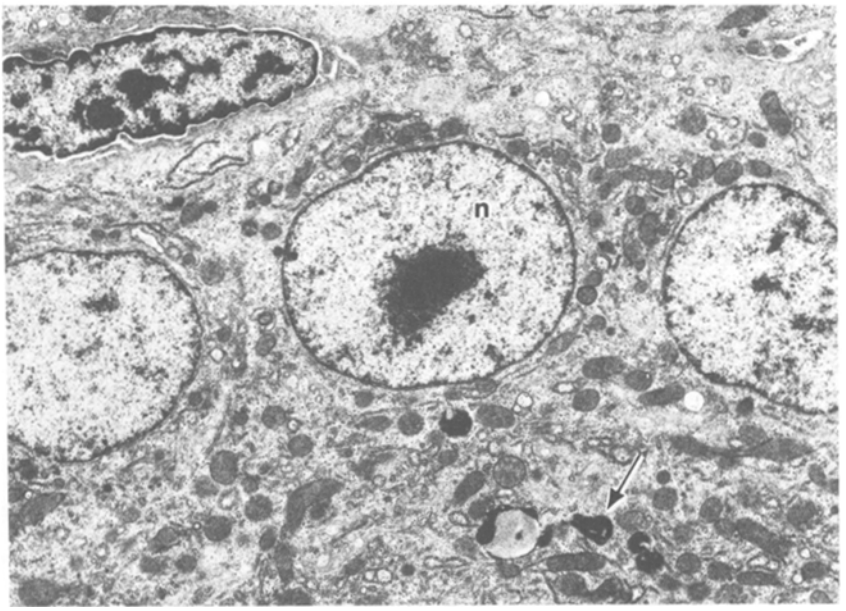

Figure 1. Transmission electron micrograph of a liver from a catfish exposed to 0 ppm arsenic for 6 months. Parenchymal hepatocyte nuclei (n) and small hemosiderin granules (arrow) are indicated. 8142 X, uranyl acetate and lead citrate post-stains.

from catfish exposed to 15 ppm arsenic for 6 months show prolifered RER, somewhat smaller nuclei, and a great increase in the size and number of hemosiderin granules (Figures 2, 3). The presence of large numbers of hemosiderin granules in parenchymal liver cells is symptomatic of iron-overloading diseases such as hemochromatosis, hemosiderosis, and hemolytic jaundice. In these diseases, ferritin (a non-heme form of soluble iron, normally present in the soluble cytoplasm)

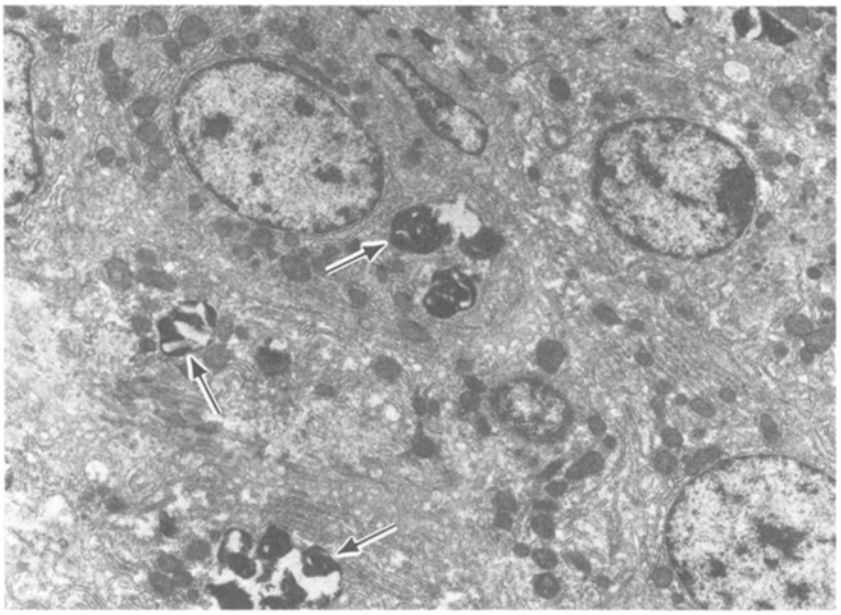

Figure 2. Transmission electron micrograph of a liver from a catfish exposed to 15 ppm arsenic for 6 months. Large hemosiderin granules (arrows) are indicated. 8142 X, uranyl acetate and lead citrate post-stains.

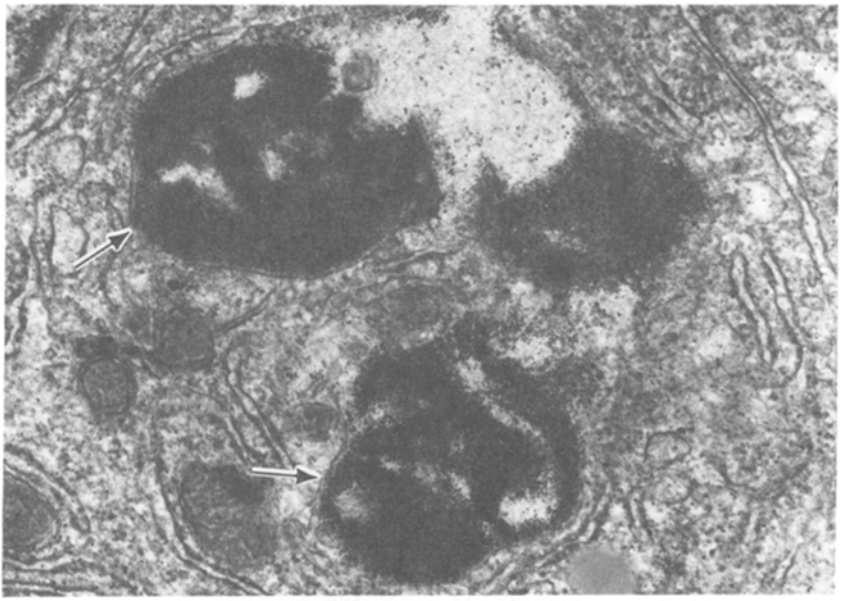

Figure 3. High magnification of hemosiderin granules (arrows) and ferritin granules dispersed throughout the cytoplasm. 39,543 X, uranyl acetate and lead citrate post-stains.

accumulates in lysosomes (ARSTILA et al. 1970; BRADFORD et al. 1969) and is degraded enzymatically to insoluble hemosiderin (CRICHTON 1971; TRUMP et al. 1973). Whereas hemochromatosis and hemosiderosis are considered disorders of iron metabolism, hemolytic jaundice is undoubtedly due to increased erythrocyte lysis. In the case of channel catfish exposed to arsenic (a known hemolytic agent), the second possibility is a more probable explanation, although the first cannot be completely eliminated from consideration since arsenicals are known to affect a number of enzyme systems.

Scanning electron micrographs of the surface of 2 µm thick frozen sections (unfixed, unstained, and uncoated) from the liver of a catfish exposed 6 months to arsenic show hepatocytes arranged in columns to form the hepatic cord (Figure 4). Nuclei were easily discerned within individual parenchymal hepatocytes, which, in addition, contain numerous cytoplasmic structures which appear in the image as bright structures. Electron probe X-ray microanalysis data (Figures 5, 6, 7) from these cytoplasmic structures indicate that all represent highly concentrated deposits of iron; 30 per cent of these cytoplasmic structures were also found to contain arsenic (L peak 1.28 keV) at a level above the minimum detectable level for our set of conditions. In these cytoplasmic structures the arsenic Kα and Kβ peaks (10.53 and 11.73 keV, respectively) were not above our detectable level.

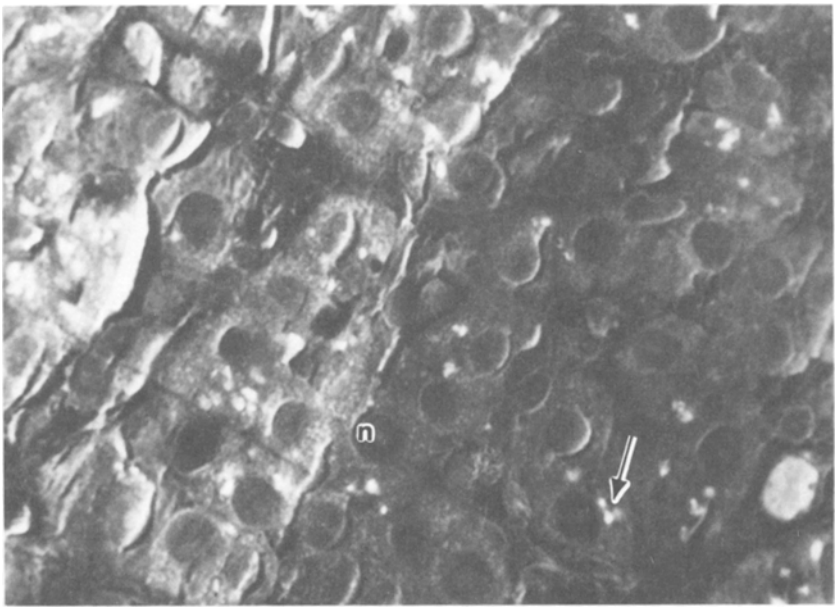

Figure 4. Scanning electron micrograph of the liver of a catfish exposed 6 months to 15 ppm arsenic. Nuclei (n) of hepatic parenchymal cells and bright cytoplasmic structures (i.e. hemosiderin granules, arrow) are indicated. 2145 X, 2 µm frozen, freeze-dried specimen, no carbon coat or post-stains.

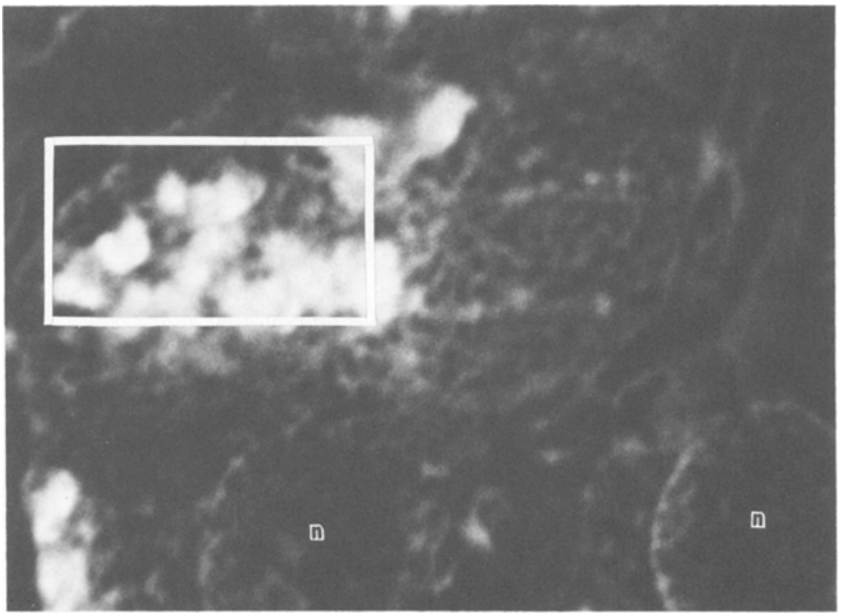

Figure 5. Scanning electron micrograph of a hepatocyte from a catfish treated 6 months with 15 ppm arsenic. The enclosed structures resemble the hemosiderin granules morphologically. Nuclei(n) are indicated. 11,440 X.

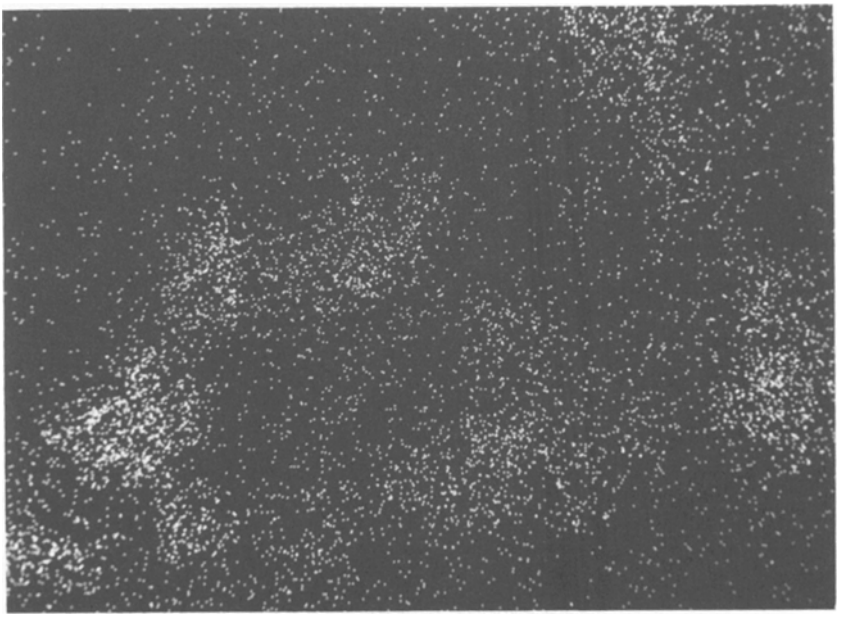

Figure 6. An iron dot map of the area enclosed in Figure 5, obtained by using Tracor Northern +AM program, which compensates for background beneath peak. A similar dot map of the continuum adjacent to the iron peak shows uniform dot distribution, hence tissue density variation does not account for dot density variation in the iron distribution map. 28,600 X.

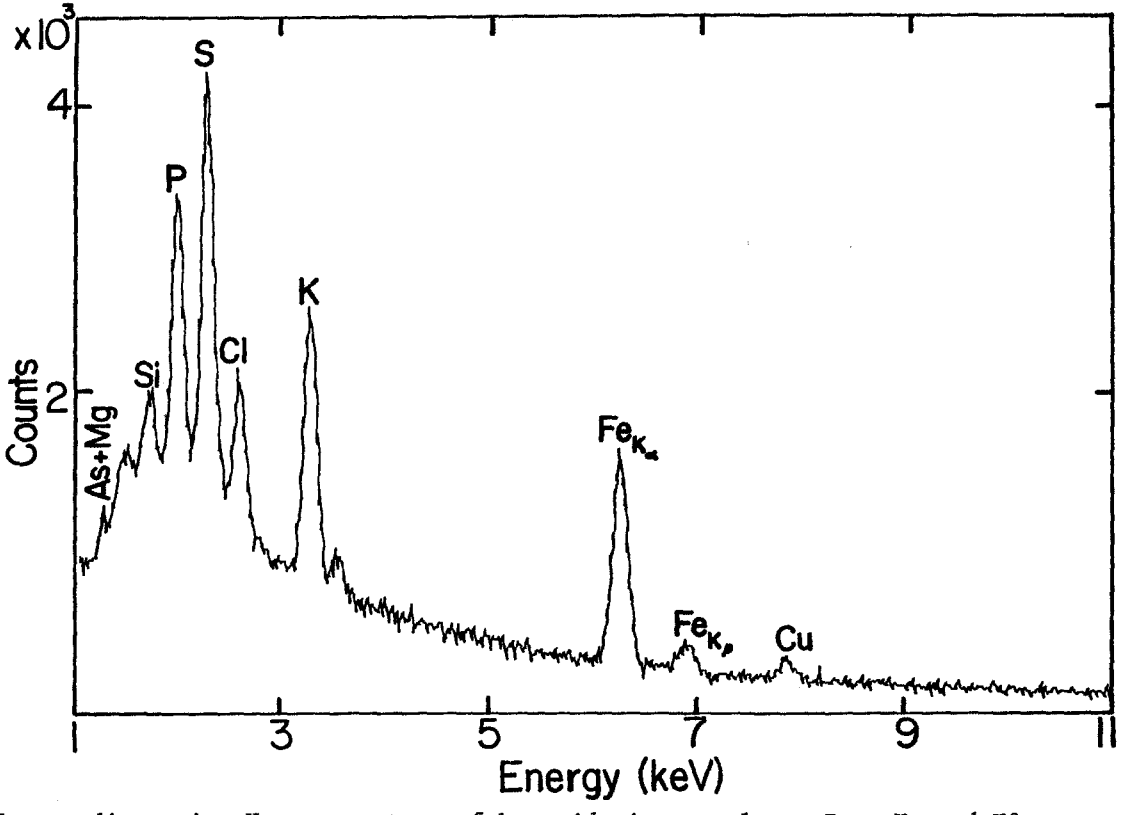

Figure 7. Energy dispersive X-ray spectrum of hemosiderin granules. Iron Kα and Kβ , as well as the arsenic Lα and Lβ (possibly superimposed over the magnesium Kα and Kβ) are indicated.

The size, shape and relative position of the cytoplasmic structures which were analyzed to determine elemental composition by XRMA were the same as the hemosiderin granules observed by CTEM. Since hemosiderin granules are iron-rich deposits, these morphological and analytical data show good correlation. The presence of arsenic (and perhaps magnesium) in these hemosiderin granules suggests an alternate mechanism for the storage of toxic metals, such as arsenic, within the hepatocyte in a less toxic, insoluble form. Such a site for metal storage in the cytopl[illegible] of catfish hepatocytes would seem less problematic in terms of overall cellular function, compared with the intranuclear arsenic inclusion observed in Lepomis cyanellus. The presence of an alternate mechanism for storage of metals and metalloids in the cytoplasm might explain the dearth of published accounts of an intranuclear arsenic inclusion.

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