Acute Effects of Methyl Mercury Toxicity in Channel Catfish (*Ictalurus punctatus*) Kidney

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The presence of methyl mercury in fish tissues is well known but the toxicity and histopathology induced by toxic dosages of this compound in various fish organs (e.g. kidney) remain to be examined in detail.

The discovery of high amounts of methyl mercury in Canadian fish (Wobesor et al., 1970) has prompted other investigations concerning mercurial contamination. Mercury levels in walleye, pumpkinseed, and lake trout ranging between 5.0 to 10.5 ppm has been reported (Fimreite et al., 1971)from Canadian lakes. The presence of mercury in these fish tissues was thought to result from industrial origin since fish were collected near sites of industrial contamination. More recently, Lockhart el al. (1972) determined the elimination rate of methyl mercury in heavily contaminated Northern Pike. They found that the rate of elimination of methyl mercury from fish organs such as kidney and liver remained essentially unchanged.

Methyl mercury occurs at relatively high concentrations in edibile species of fish from many parts of the North American continent (Bache et al., 1971). Total mercury levels in largemouth bass have been reported (Knight and Herring, 1972) from the Ross Barnett Reservoir in Mississippi and ranged from 0.05 to 0.74 ppm. Also catfish from many areas of Mississippi contain measurable mercury levels (Cotton and Herring, 1973), ranging from 0.01 to 0.37 ppm. These residues of mercury in fish are often present largely as highly toxic methyl mercuric salts (D'Itri, 1972) and therefore methyl mercuric chloride was utilized in this study. The present research was conducted to determine the histopathologic alterations that accompany acute methyl mercury intoxication in the channel catfish kidney at the relatively high dosage of 12 ppm methyl mercury since this concentration has been shown (Kendall, 1972) to induce alterations that can be visualized at the light microscopic level.

Methods

Fish

Channel catfish (250) measuring 25-35 cm in length were obtained from a dealer in Shelby Co., Kentucky and kept in 110 gallon glass aquaria. Aquarium water was maintained at 23°, well aerated and filtered through glass wool and charcoal.

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Bulletin of Environmental Contamination & Toxicology, Vol. 13, No. 5 © 1975 by Springer-Verlag New York Inc.

Methyl mercuric chloride (CH_3HgCl) was mixed in an aqueous solution of 5mM sodium carbonate (Norseth and Clarkson, 1970) to facilitate dissolution. Fish were injected intraperitoneally with the above solution containing 12 ppm methyl (15 ppm methyl mercuric chloride). This dosage level has been reported as the LD_{50} for pike (Miettinen, 1970) and was selected here as a dose with which one could expect tissue alterations. Controls received injections of 5mM sodium carbonate without CH_3HgCl.

Following injections, fish were killed at 24 hour intervals through 96 hours and subsequently processed for atomic absorption spectrophotometrical, enzyme histochemical, and histological analysis (see below).

Atomic Absorption Spectrophotometry

Organ concentrations of mercury were quantified using a Perkin-Elmer flameless, atomic absorption spectrophotometer. Portions of kidneys from control and experimental fish were excised and placed in dilute (3%) nitric acid in screw top vials and 5 ml of concentrated sulfuric acid was added to each vial and tissue hydrolysis was continued until solutions were clear. Mercury content was analyzed using the method of Uthe, Armstrong, and Stainton (1970).

Enzyme Histochemical Techniques

Fresh frozen sections (10 μ) from control fish were cut in an International freezing microtime and affixed to precooled albuminized slides. Succinic dehydrogenase activity was localized (Nachlas *et al.*, 1957) using the tetrazolium salt Nitro-BT and sodium succinate as substrate. For comparison, ten sections were exposed to the incubation solution without CH_3HgCl and ten were exposed to the incubation solution plus 15 mg/L CH_3HgCl. Incubation time was 60 minutes at room temperature (23°C).

Histologic techniques

Fish were stunned by a blow to the head and kidneys removed and placed in Bouin's fixative for 18 hours. Tissues were subsequently washed for 8 hours in running tap water, dehydrated in ethanol, cleared in xylene, and embedded in paraffin (56° Paraplast) using a Lipshaw tissue processor. Sections (6-7 μ) were dried and stained using the periodic-acid-Schiff's reagent and hematoxylin (PAS+H) according to McManus and Mowry (1960). Slides were dehydrated, cleared in xylene, and mounted in Permount.

Statistics

Statistical analysis of variance using the Newman-Keuls procedure (Winer, 1962) was performed on mercury data with the aid of an IBM 1130 computer. To determine significance, results were compared to values in an f-table at the $P < 0.01$ probability level.

Results

At 24 hours all catfish injected with CH_3HgCl could easily be distinguished from the control group, the former having greatly distended abdomens. When opened, the abdominal cavaties were filled with a reddish-yellow, serous exudate. The accumulation of this fluid seemed to increase over the experimental time period (96 hours). In addition, mesenteric vessels were distended and appeared inflammed.

Mercury date (Table 1) revealed high uptake of mercury in kidneys from 24 hours (mean value= 51.03± 15.5), 48 hours (mean value= 38.26± 6.4), 72 hours (mean value= 39.48±4), 96 hours (mean value= 14.24± 5.2). These results were significantly ($P < 0.01$) different from control values.

Table 1

Mean concentration (ug/g tissue) of Mercury in Selected Intervals Following a Single Intraperitoneal Injection of 15 mg/kg CH_3HgCl.

No. of fish in each group	Kidney	
50	Controls	0.25±.2
15	24 hours	51.03±15.5
15	48 hours	38.26±6.4
15	72 hours	39.48±4.0
15	96 hours	14.24±5.2

± = Standard Deviation

Severe glomerular and tubular alterations were evident at 24 hours (fig. 1). Glomeruli were swollen and heavily stained with PAS reaction product and flomerular basement membranes were prominent. At 48 hours (fig. 2), glomeruli did not stain as intensely as those in fig. 1 and basement membranes appeared even thicker than at 24 hours. Desquamation of tubular epithelial cells was quite noticeable and proceeded to extreme desquamation (fig. 3) by 72 hours. Glomeruli lost their distinctive morphology and were not readily discernible at 96 hours (fig. 4). By 96 hours a widespread loss of cellularity was evident with complete desquamation of cells into tubular lumens and tubular necrosis being common features. Microscopic sections from unexposed controls appeared normal.

Enzyme histochemical results for succinic dehydrogenase activity revealed marked inhibition (fig. 6) when compared to controls (fig. 5). This inhibition was manifested as focal diminution of enzyme activity.

No histopathologic alterations were observed in renal tissue from control fish.

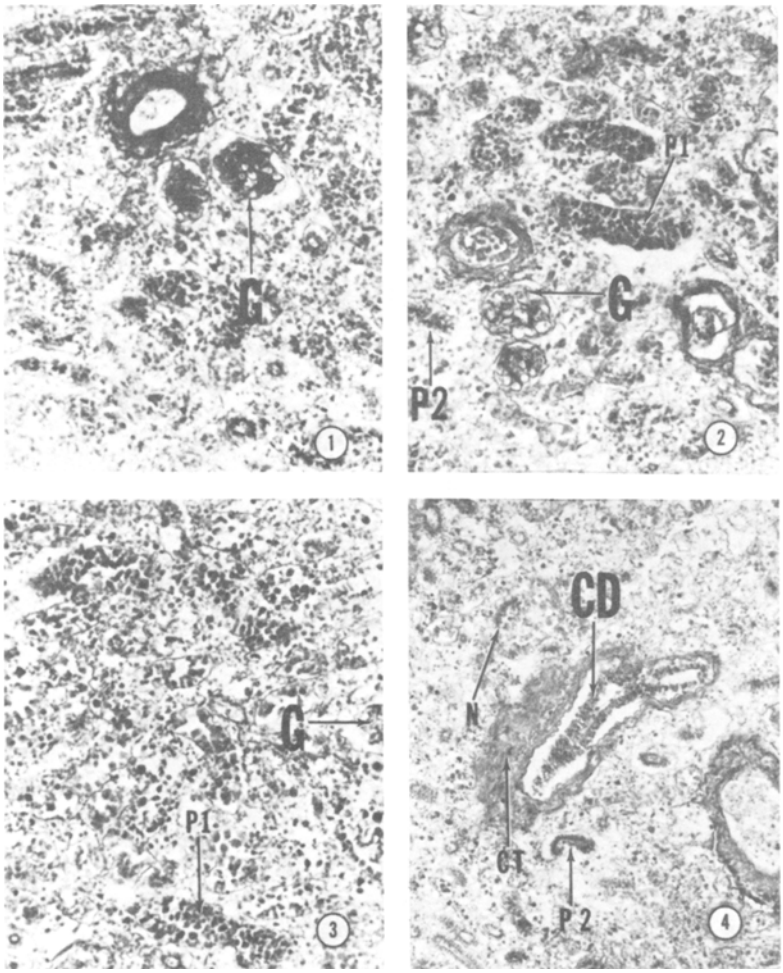


Plate 1

Captions for Illustrations

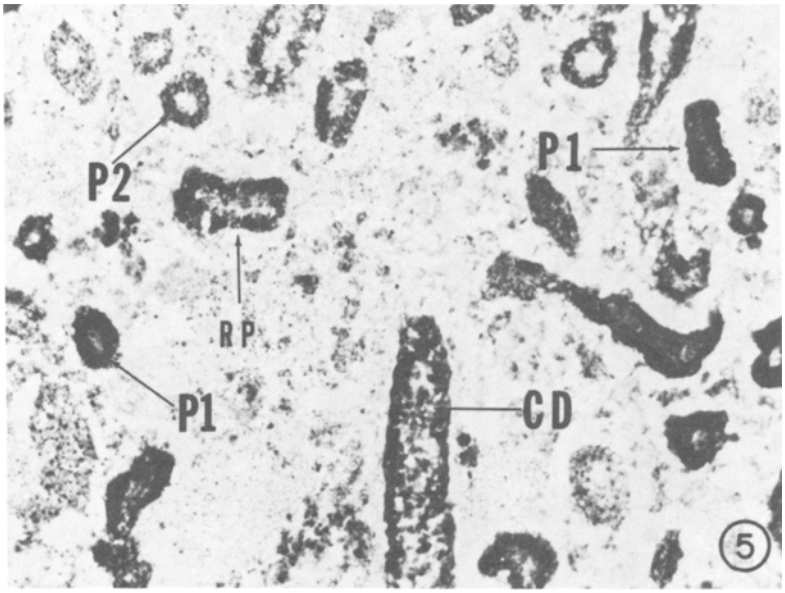
Figures 1-4. Histopathological effects of methyl mercury toxicity in channel catfish kidneys. Fish received a single intra-peritoneal injection of 15 ppm methyl mercuric chloride.

Fig. 1 At 24 hours glomeruli (G) are heavily stained with PAS reaction product and glomerular basement membranes are prominent. Some desquamation of tubular epithelium can also be seen. PAS+H, X 300.

Fig. 2 At 48 hours glomeruli (G) do not stain as intensely as at 24 hours and some loss of cells is evident. Most prominent are desquamation of PAS positive cells into lumens of proximal tubules (P1). These cells appear very dark in this photomicrograph. Tubular epithelium of the second proximal tubule (P2) is not nearly as desquamated as P1. PAS+H, X 300.

Fig. 3 At 72 hours fewer cells are observed and glomeruli (G) are degenerated or been excreted and some of the very last cells to be desquamated are those of the collecting duct (CD) shown in the center of the figure. Also, the heavy layer of connective tissue (CT) surrounding the collecting duct appears thickened and a necrotic tubule (N) is visible. Desquamating epithelium of the second proximal tubule is also shown (P2). PAS+H, X 300.

Fig. 4 At 96 hours tubular and hematopoietic cells have degenerated or been excreted and some of the very last cells to be desquamated are those of the collecting duct (CD) shown in the center of the figure. Also the heavy layer of connective tissue (CT) of the collecting duct appears thickened and a necrotic tubule (N) is visible. Desquamating epithelium of the second proximal tubule (P2) is also shown. PAS+H, X 200.



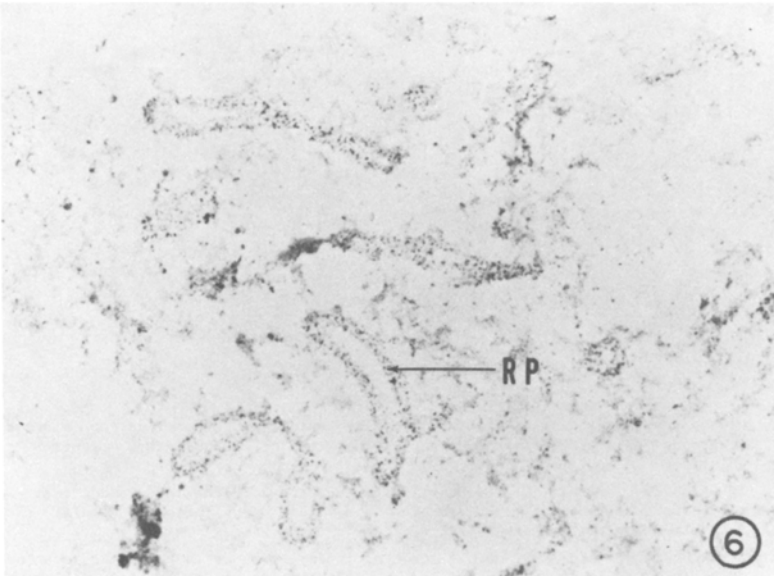


Plate 2

Captions for Illustrations (Cont.)

Figures 5 and 6. A comparison of succinic dehydrogenase activity in cryostat sections of catfish kidney exposed (in vitro)and not exposed to 15 mg/L methyl mercuric chloride.

Fig. 5 Unexposed control showing succinic dehydrogenase reaction product (RP) in renal tubules. Heaviest reaction product is seen in the first proximal tubule (P1) and collecting duct (CD). Less activity is seen in the second proximal tubule (P2). Nachlas method. X 500, enlarged 2X.

Fig. 6 Succinic dehydrogenase reaction product (RP) following exposure to 15 mg/L methyl mercuric chloride in vitro. Notice focal diminution of enzyme activity. Nachlas method. X 500, enlarged 2X.

Discussion

The renal tubular morphology of channel catfish has been described by Kendall and Hinton (1974). Those observations formed the baseline for comparison with the altered morphology seen in the present study.

For centuries mercury has been recognized as a nephrotoxic agent. Taylor and Pavy (1860) first described structural changes in kidney tissues following mercury poisoning in mammalian systems. Subsequently many investigators have studied mercury induced nephrosis (Taylor, 1965). Taylor studies alterations induced by graded sublethal dosages of mercuric chloride in rats. When 5.0 ppm mercury was administered, desquamation of cells into tubular lumens was observed. In the present study similar cellular desquamations occurred in catfish kidney at 48 hours after exposure to methyl mercury. Mercury analysis data in the present study indicated a fall in mercury concentration at 96 hours which paralleled the striking loss of cellularity at this time, the conclusion being that the kidney was "excreting itself" which accounted for the decline in mercury concentration. Backstrom (1969) reported a high uptake of methyl mercury in kidneys of salmon that were injected with methyl mercury intravenously. A similar observation for catfish was noticed in this study.

Mercurials are considered highly toxic to fish (Doudoroff and Katz, 1953; Alabaster, 1958) but fish from Sweden have shown no toxic symptoms in spite of their relatively high mercury content (Backstrom, 1969). However, toxic effects have been described under experimental conditions (Carpenter, 1927; Boetius, 1960). Changes brought about by feeding methyl mercury to pike, have been reported (Miettinen et al., 1969). These included damage to pseudobranch, liver, and kidney. Alteration in the kidney included tubular necrosis and the liver contained necrotic areas. In addition to the renal alterations observed in methyl mercury treated channel catfish, hepatic alterations were also observed and will be reported in a forth-coming paper.

Succinic dehydrogenase (SDH) has been localized histochemically in rat kidney tubules (Mustakallo and Telka, 1953) and channel catfish kidney (Kendall, 1972) and the dehydrogenase tetrazolium techniques have often been applied in studies of alteration due to the well known inhibitory effects on SDH by heavy metals (Barron and Kalnitsky, 1947). Numerous mitochondria in the distal segments and collecting ducts of fish kidney (Hickman and Trump, 1969) account for the large amount of SDH activity in these tubules. Bahn and Longley (1956) showed in the rat that a mercurial diuretic decreased renal SDH activity in the outer zone of the renal medulla. However, since the catfish kidney contains no zones similar to the rat kidney, the decrease in SDH activity observed in the present study included the entire kidney. Yoshino et al., (1966) observed a significant decrease in SDH activity in rat brain poisoned with an alkylmercurial compound. Also, a selective

inhibition of protein synthesis was noticed and this may have accounted for the observed toxicity. A similar inhibition of SDH was noted in this study and may reflect an equivocal toxic response due to inhibition of protein synthesis or perhaps direct inhibition of enzyme by mercury.

Taylor (1965) observed desquamation of rat renal tubular epithelium at 12 hours when 5.0 mg/kg mercury was administered. Similarily, catfish epithelium was seen to desquamate in response to methyl mercury, although a higher dosage (12.0 mg/kg mercury) was utilized in this study.

Focal lesions in the rat proximal convoluted tubules have been induced by mercuric bichloride poisoning (Oliver, McDowell, and Tracy, 1951). They termed this condition nephrotoxic nephrosis and attributed the lesion to direct toxicity of mercury on tubular epithelium More recently, Wachstein and Meisel (1957) localized the mercury nephrotoxic lesion to more terminal portions of the proximal convoluted tubules. The classical histological techniques for studying mercurial induced nephrosis were reviewed by Rodin and Crowson (1962). Using these techniques in channel catfish kidneys that were subjected to methyl mercury intoxication, generalized necrosis was noticeable at 96 hours but probably various specific tubular segments were affected sooner than this time.

In humans, thickenings of glomerular basement membranes occurs in membranous glomerulonephritis, systemic lupus erythematosus, diabetes mellitus, and arteriosclerosis (Robbins and Angell, 1971). The thickened glomerular basement membranes resulting from methyl mercury exposure observed in this study may have resulted from the direct toxic action of mercury on renal cells or as secondary contributory pathologies induced by methyl mercury on other organs (e.g. pancreas) that in turn may have produced the glomerular thickenings.

Finally, by demonstration of tissue alteration subsequent to exposure to aquatic pollutants such as mercury, histologic and histochemical assessments prove the toxic nature of these compounds (Hinton, Kendall, and Silver, 1973). In addition, the extent of alteration may become the basis for prognosis of effects of various concentrations of these pollutants.

Conclusions

The results of this study have shown that in the channel catfish (Ictalurus punctatus) :

1. a single intraperitoneal injection of 15 mg/kg methyl mercuric chloride caused deposition of mercury in the kidney;

2. inhibition of histochemically localized succinic dehyrogenase activity;

3. marked pathology as evidenced by necrosis of renal tubular cells and increased thickening of renal glomerular basement membrane.

Acknowledgements

Support for this research was partially derived from funds provided by the U.S. Department of the Interior, Office of Water Resources Research, as authorized under the Water Resources Act of 1964, contract No. 14-31-0001-3217. I would also like to thank Dr. David E. Hinton for helpful suggestions and support offered during the tenure of this research and preparation of the manuscript.

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