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

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Methyl mercury has been discovered in fish tissues world-wide and although mercurial presence in the environment has been well established, the histopathological effects of toxic dosages in fish organs (e.g. liver) remains to be examined in detail (KENDALL 1975). Fishes are obviously the most important source of methyl mercury in human food and the daily intake in fish-eating populations corresponds directly with amounts of food consumed daily (BERGLUND et al. 1971 and MCDUFFIE 1971).

According to BACHE et al. (1971), methyl mercury occurs at relatively high concentrations in edible fish species from many regions of North America. Catfish from many areas of the state of Mississippi contain mercury levels ranging from 0.01 to 0.37 ppm. Mercury residues in fish are often present as highly toxic mercuric salts (D'Itri 1972). For this reason, methyl mercuric chloride was used in the present study.

It has been suggested (HINTON et al. 1973) that tissues of fish might serve as sensitive indicators of aquatic pollution, namely methyl mercury contamination. Various enzymes (succinic dehydrogenase, acid, and alkaline phosphatases) in catfish liver and kidney have been shown to be very sensitive to methyl mercury toxicity (KENDALL 1972). In addition, catfish kidneys are severely damaged by LD_{50} dosage levels of methyl mercury (KENDALL 1975).

The purpose of this study, then, was to describe the sequential toxic effects in catfish liver produced by an acute LD_{50} dosage of an organic mercurial compound (methyl mercuric chloride) since it has been estimated that 85-95% of organic mercury exists in the methylated form in fish (JARVENPAA et al. 1970).

Methods

Fish

Channel catfish (110) measuring 25-35 cm in length were obtained from a dealer in Shelby Company Kentucky and housed in 110 gallon glass aquaria. Aquarium water was maintained at 23°C, well aerated and filtered through glass wool and charcoal. Fish were allowed to become adapted to these artificial conditions for three months after which the experiment ensued.

Methyl mercuric chloride (CH_3HgCl) was mixed in an aqueous

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solution of 5mM sodium carbonate (NORSETH and CLARKSON 1970) to facilitate dissolution. Fish were injected intraperitoneally with the above solution containing 12 ppm mercury (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 the injections, fish were killed at 24 hour intervals through 96 hours and their livers were subsequently processed for atomic absorption spectrophotometrical and histological analysis (see below).

Atomic Absorption Spectrophotometry

Liver concentrations of mercury were quantitated using a Perkin-Elmer flameless, atomic absorption spectrophotometer. Livers from control and experimental fish were excised and placed individually 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, ARMONSTRONG and STAINTON (1970).

Histological Techniques

Fish were stunned by a blow to the cephalic region and livers 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^{o} paraplast) using a Lipshaw tissue processor. Sections (6-7 μ) were dried and subsequently 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

Mercury analysis data (Table 1) revealed high uptake and increasing concentration of mercury in livers from 24 hours (mean value = 59.0 ± 27.2), 48 hours (mean value = 81.0 ± 14.0, 72 hours (mean value = 124.0 ± 25), to 96 hours (mean value = 157.0 ± 62.0). These results were significantly different ($P < 0.01$) from control values.

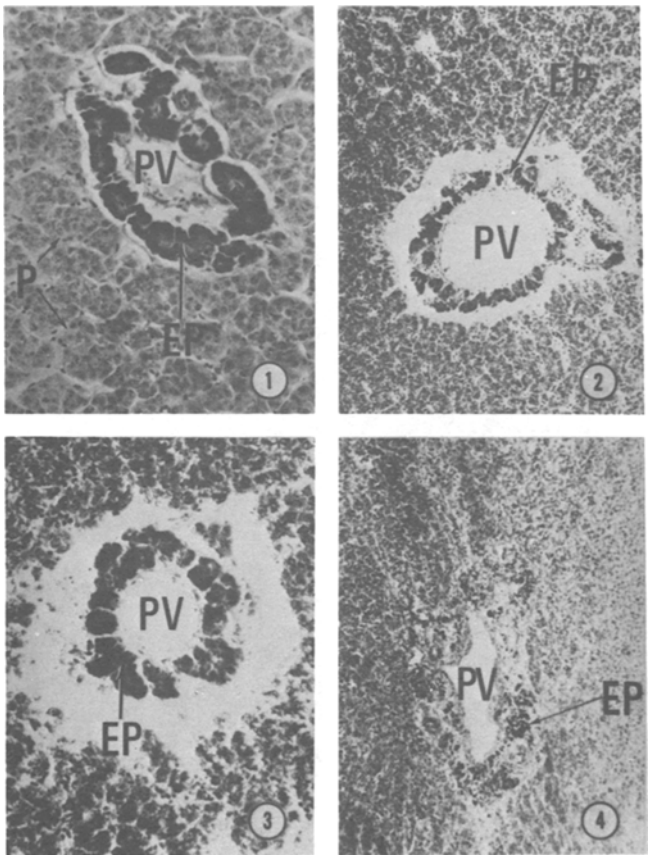

PLATE 1

Fig. 1 Control receiving no methyl mercuric chloride. Note the portal vein (PV) surrounded by exocrine pancreas (EP) and parenchymal cells (P). Hematoxylin, X 375.

Figs. 2-4 Histopathological effects of methyl mercury toxicity in channel catfish liver. Fish received a single intraperitoneal injection of 15 ppm methyl mercuric chloride.

Fig. 2 At 24 hours the first histological alterations include apparent shrinkage of exocrine pancreatic cells and the appearance of a circumferential around these cells. Hematoxylin, X 175.

Fig. 3 At 48 hours the space enlarges and cell borders appear disorganized compared to control tissue (fig. 1). Hematoxylin, X 400.

Fig. 4 At 72 hours the space around the exocrine pancreatic tissue is necrotic and only PAS positive cell remnants remain visible. PAS + H, X 175.

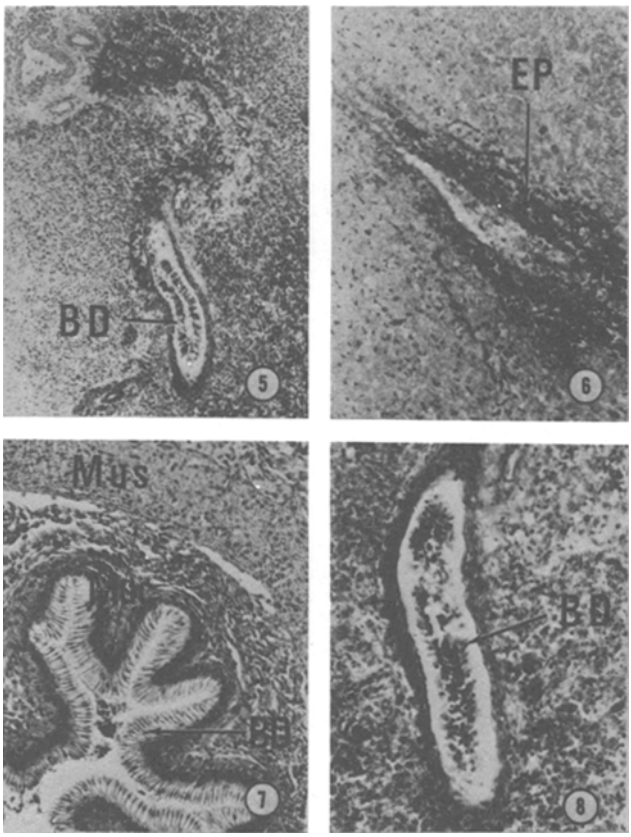

PLATE 2

Captions for Illustrations

Fig. 5 At 96 hours the bile duct epithelium (BD) desquamates into the lumen. Hematoxylin, X 150.

Fig. 6 At 96 hours the exocrine pancreatic tissue (EP) is hard to identify with cell remnants and pycnotic nuclei being present. PAS + H, X 400.

Fig. 7 Control tissue. Section of the bile duct with epithelium (BD) and circumferential mucosa (Muc) and muscularis (Mus). Tall columnar epithelium lines the bile duct. Hematoxylin, X 350.

Fig. 8 Desquamated bile duct epithelium (BD) is shown from a 96 hours experimental fish (compare with fig. 7). Hematoxylin, X 400.

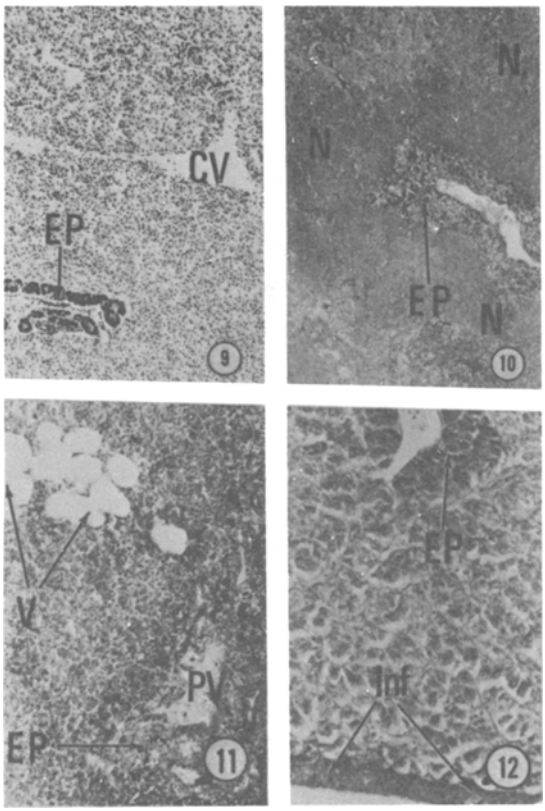

PLATE 3

Fig. 9 Control receiving no methyl mercuric chloride. Note the exocrine pancreatic tissue (EP) surrounding a branch of the portal vein. Branches of central vein (CV) lies just above. Hematoxylin, X 150.

Fig. 10 Section of liver from a blanched (whitish) area. At 96 hours necrosis (N) of parenchymal cells radiates from a branch of the portal vein around which some cellular remnants of the exocrine pancreas (EP) can be seen. Hematoxylin, X 150.

Fig. 11 At 96 hours some vacuolated regions (V) are found, usually near branches of the portal vein (PV). Necrotic, disorganized cells of the exocrine pancreas (EP) are also seen. PAS + H, X 150.

Fig. 12 At 96 hours the hepatic capsular surface displays a layer of inflammatory exudate (Inf). Various types of blood cells and fibrin are found in this PAS positive layer. PAS + H, X 400.

TABLE 1

Mean concentration (μg/g tissue) of mercury at selected intervals following a single intraperitoneal injection of 15 mg/kg CH_3HgCl.

No. of fish in each group	Liver Group	(μg/g) Concentration
50	Controls	0.10 ± 0.02
15	24 hours	59.0 ± 27.2
15	48 hours	81.0 ± 14.0
15	72 hours	124.0 ± 25.0
15	96 hours	157.0 ± 62.0

± = standard deviation

All catfish injected with CH_3HgCl could be distinguished from members of the control group as early as 24 hours. The experimental group members had greatly distended abdomens and when these were incised, revealed a reddish-yellow, serous exudate. Generally this fluid seemed to increase over the experimental time period (96 hours). Mesenteric vessels appeared inflammed and distended.

At 24 and 48 hours the livers from experimental fish, upon gross inspection, were similar to controls. But at 72 and 96 hours livers from experimental fish revealed blanched necrotic foci on their outer capsular surfaces and this was not observed on livers from control fish. Bile accumulation was remarkable since the gall bladder was greatly distended with copius amounts of biliary fluid.

Numerous histological alterations were noticed when compared to controls (figs. 1 and 9). The hepatic parenchyma of experimentals was not affected as much as the more sensitive exocrine pancreatic tissue. Thus at 24 hours (fig. 2) exocrine pancreatic tissue appears shrunken and a space becomes prominent between exocrine pancreatic cells and adjacent hepatic parenchyma. At 48 hours this space apparently widens and exocrine pancreatic cells directly adjacent to it appear disorganized and degenerated when compared to the control (fig. 1). At 72 hours (fig. 4) the exocrine pancreatic tissue has completely lost its former distinctive cellularity and only PAS positive cell remnants are present. By 96 hours (figs. 5, 6 and 8) the exocrine pancreative tissue has become necrotic making identification difficult, and this is termed periportal necrosis.

Another striking affect of methyl mercury intoxication was the desquamation of bile duct epithelium into the duct lumen at

96 hours (figs. 5 and 8). Normally the bile ducts and ductules are lined with tall columnar epithelium (fig. 7).

An area of necrosis is shown in fig. 10 from a blanched (whitish) region of the liver. Remnants of the exocrine pancreatic tissue can also be detected near the center of the necrotic region. In addition to cellular necrosis observed at 96 hours, some parenchymal regions contained several vacuoles (fig. 11).

A region of inflammation located at the capsular surface (fig. 12) almost completely encased the liver. This suppurative, inflammatory exudate consisted of cells and fibrin that were PAS positive.

Discussion

Various tissues of fish organs have not been adequately described in the literature so as to form a basis for comparison to experimentally-induced histological alterations. Fortunately, channel catfish liver has been described at both the light and electron microscopic level (KENDALL and HAWKINS 1975) and provides a reference for the present study. The channel catfish liver, similar to other fish species, consists of laminae of dual-plated muralium and, unlike those of many species, dispersed exocrine pancreatic tissue that circumscribes the portal vein, its tributaries, hepatic artery, and its branches.

BÄCKSTRÖM (1969) injected speckled trout with 0.1 mg/kg methyl mercuric nitrate and reported dilated bile ducts as the only pathologic change in the liver. MIETTINEN et al. (1970) administered protein bound methyl mercury to pike at a lethal concentration of 75.5 mg/kg and noticed abnormal coloration and small green, necrotic areas in the liver. The former study used a different compound than the present study and was administered at an extremely low dosage level, therefore, it is not surprising to find such a mild change. The latter study used an extremely high dosage (approximately 6 times the dosage used in the present study) and since the dosage was lethal to all pike, it is not surprising that liver necrosis occurred also.

High organic mercury levels in livers have been reported in poultry (SWENSSON and ULFVARSSON 1968) and mice (BERLIN and ULLBERG 1963) after injection of phenyl mercurials. Mercury has been shown by SWENSSON and ULFVARSSON to present a pronounced periportal localization in rats. This is consistent with observations in mice (BERLIN and ULLBERG 1963) and rabbits (FRIBERG et al. 1957). BÄCKSTRÖM (1969) suggested that the portal localizations may be correlated to direction of blood and bile flow in the liver. Since liver necrosis in catfish also was located in a periportal location the explanation offered by BÄCKSTRÖM for mammalian species may also be true in catfish.

Generally, mercurials are considered highly toxic to fish (DOUDOROFF and KATZ 1953; ALABASTER 1958) but according to

BÄCKSTRÖM (1969) fish from Sweden have shown no toxic symptoms in spite of their relatively high mercury content. 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). Tissue damage included renal tubular necrosis and areas of necrosis were observed in the liver. Similar liver pathology was observed in the present study and, in addition, exocrine pancreatic tissue in the catfish hepatopancreas demonstrated the earliest histopathologic change.

Conclusions

The results of this study have demonstrated that in the channel catfish (Ictalurus punctatus) a single intraperitoneal injection of 15 mg/kg methyl mercuric chloride caused:

1. Deposition of mercury in the liver.

2. Accumulation and concentration of mercury over the time period of 96 hours.

3. Marked pathology as evidenced by necrosis of exocrine pancreatic and parenchymal cells at 72 and 96 hours.

4. Inflammation at the hepatic capsular surface.

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Bibliography

ALABASTER, J. S.: Proc. 4th Brit. Weed Control Conf. 1-2 (1958).

BACHE, C. A., W. GUTENMANN, and D. LISK: Science, 172: 951 (1971).

BÄCKSTRÖM, J.: Acta Pharmacol. Toxicol., 27 (3): 1 (1969).

BERGLUND, F., M. BERLIN, G. BIRKE, U. VON EULER, L. FRIBERG, B. HOLMSTEDT, E. JONSSON, C. RAMELL, S. SKERFVING, A. SWENSSON, and S. TEJNING: Nord. Hyg. T. Supplement 4 (1971).

BERLIN, M. and S. ULLBERG: Archs. Environ. Health, 6: 589 (1963).

BOETIUS, J.: Medd. Banmarks Fiskeri-og Havunderogelsser, 3: 93 (1963).

CARPENTER, K. E.: Brit. J. Exptl. Biol., 4: 378 (1927).

D'ITRI, F.: CRC Press, The Chemical Rubber Co., Cleveland, Ohio (1972).

DOUDOROFF, P.: In M. Brown: The Physiology of Fishes. Acad. Press, New York, 403 (1957).

FRIBERG, L., E. ODEBLAD, and S. FORSSMAN: A.M.A. Arch. Ind. Health, 16 (1957).

HINTON, D. E., M. W. KENDALL, and B. B. SILVER: In Biological Methods for the Assessment of Water Quality. American Society for Testing and Materials. STP 528, Los Angeles, 194 (1973).

JARVENPAA, T., M. TILLANDER, and J. M. MIETTINEN: Suomen Kemistileht: B43: 439 (1970).

KENDALL, M. W.: Ph.D. Thesis. 101 p. Univ. of Louisville, Louisville, Kentucky, University Microfilms; Ann Arbor, Michigan (1972).

KENDALL, M. W.: Bull. Environ. Cont. Toxicol., 13 (5): 570 (1975).

KENDALL, M. W. and W. E. HAWKINS: J. Fish Res. Bd. Can., 32: 1459 (1975).

MCDUFFIE, B.: Binghampton News. Jan. 13, 1971.

MCMANUS, J. F. A. and R. W. MOWRY: Staining Methods, Histologic and Histochemical. Paul Hoeber, Inc., New York, NY (1960).

MIETTINEN, V., Y. OHMOMO, M. VALTONEN, E. BLANKENSTEIN, K. RISSANEN, M. TILLANDER, and J. K. MIETTINEN: Fifth R.I.S. Symp. 1969, Helsinki, Finland (1969).

MIETTINEN, J. K., M. HEYRAUD, and S. KECKES: FAO FIR: MP:70: E-90. Rome, November (1970).

NORSETH, T. and T. CLARKSON: Biochem. Pharmacol., 19: 2775 (1970).

SWENSSON, A. and U. ULFVARSSON: Acta Pharmacol. Toxicol., 3: 259 (1968).

UTHE, J. F., F. A. J. ARMSTRONG, and M. P. STAINTON: J. Fish. Res. Bd. Canada, 27 (4): 805 (1970).

WINER, B. J.: Statistic Principles in Experimental Design. McGraw-Hill, New York, 80 (1962).