with a chloride ion concentration of less than 1 mg/liter, nitrite is extremely toxic. The oxidation of ferrohemoglobin to ferrihemoglobin by nitrite or a derivative of nitrite is observed in nitrite toxicity. Under normal conditions, there is a change in the color of the gill tissue and the blood to a characteristic chocolate brown color. In our work, we have also experienced high mortality at moderate levels of ferrihemoglobin with an absence of blood color change. This suggests that a second mechanism may be operative. At this time, we are looking into two possible mechanisms: one involving interference in the final step involving electron transfer in the respiratory enzymes, and the other the effect of stress on the production of mineralocorticosteroids. In the case of the latter, elevated blood bicarbonate levels would result in alkalemia with its associated impairment of respiration.

Research is also underway on the problems involved during smoltification of steelhead trout in a water reuse system. Although work in this area is still in the exploratory stage, available data suggests that the problem is related to a lack of synchronization between increased hormonal activity associated with smoltification and activation of the Na+K ATPase system in the gill tissue.

In conclusion, it appears that environmental stress factors have a far greater influence on normal growth and development than previously recognized. High density culture in water reuse systems, although theoretically possible, is not without considerable risk, and the risk will not be minimized until we have a better understanding of the relationship between environmental factors and the physiology of the cultured species.

MFR Paper 1349. From Marine Fisheries Review, *Vol. 40, No. 10, October 1978. Copies of this paper, in limited numbers, are available from D822, User Services Branch, Environmental Science Information Center, NOAA, Rockville, MD 20852. Copies of* Marine Fisheries Review *are available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 for $1.10 each.*

MFR PAPER 1350

PCB-Induced Alterations in Teleost Liver: A Model for Environmental Disease in Fish

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Man's population and continued economic and industrial development has led to increasing exposure of the aquatic environment to exogenous compounds. Alteration in structure/function of tissues arising from exposure to these pollutants comprises our working definition of environmental diseases of fish. The response of fish tissues to environmental pollutants may take either of two forms. In the first, there is acute lethal injury. This may be expected to occur after exposure to extremely toxic substances. The second form occurs following exposure to chronic sublethal concentrations of pollutants. This leads to an altered steady state, compatible with life, where changes in structure at the tissue, cellular, and organelle level can be correlated with alterations in function. Those pollutants which lead to the latter form of injury are noted by their persistence in the environment and their bioaccumulation.

The ability of fish to metabolize compounds can, to a large extent, mediate the form of injury which results. The primary locus of enzymes which metabolize foreign substances is the hepatic microsomal mixed-function oxidative system (MFOS). This system is capable of the detoxification of noxious substances and the activation of potential carcinogens (Dallner, 1963).

The polychlorinated biphenyls (PCBs) are important industrial compounds which have been used in paints, immersion oils, electrical capacitors, and heat exchange systems. Their presence and persistence in the aquatic environment have been documented

(Gustafson, 1970). From studies on mammalian liver, it is known that PCBs cause marked induction of enzymes in the MFOS (Bruckner et al., 1974; Litterst et al., 1972). Morphologic studies have shown hepatocytomegaly, proliferation of smooth endoplasmic reticulum, and lipid accumulation (Kasza et al., 1976; Kimbrough et al., 1972). Based upon a limited number of biochemical studies, it appears that the MFOS of teleost liver undergoes a similar response to PCB although the degree of enzyme induction is less (Hill et al., 1976; Lidman et al., 1976). Morphologic findings in PCB-exposed fish have not been previously reported. The purpose of this report is to present correlated morphologic and biochemical data in channel catfish liver following exposure to PCB.

Mixed-sex channel catfish with an average weight of 165 gm were obtained from the Agricultural Engineering Department at the University of Maryland, College Park. For each experiment, control and PCB-exposed fish were kept in 20-gallon glass tanks with noncirculating but aerated water at a temperature of 12°-15°C. Water was changed daily. Fish were starved during the exposure period. For acute exposure, fish were given seven daily intraperitoneal injections of PCB (Aroclor 1254, Monsanto[1]) in mineral oil (50 mg/kg body weight). Controls were given mineral oil only. For subacute exposure (21 days), PCB (1,000 mg/kg body weight) in gelatin capsules was administered by gastric insertion.

Following exposure, fish were stunned by a blow to the head and the liver was removed, blotted, and weighed. Tissue was minced in ice cold 0.05M Tris-1.15 percent KCl buffer, pH 7.4. Three to four washings in buffer were employed to remove the blood. The entire liver was placed in fresh ice cold buffer and a 25 percent w/v homogenate was made by four passes with a teflon pestle at 200 rpm. The homogenate was sedimented at 10,000 *g* for 15 minutes, and the supernatant was centrifuged at 100,000 *g* for 60 minutes. The resulting microsomal pellet was resuspended in Tris-KCl buffer to a final concentration of 0.5 gm/ml. Standard methods were used to quantify the following microsomal components (incubation temperatures were lowered to 27°C): Cytochrome P-450, cytochrome b_5, NADPH-cyt-c reductase, microsomal protein, and aminopyrine demethylase.

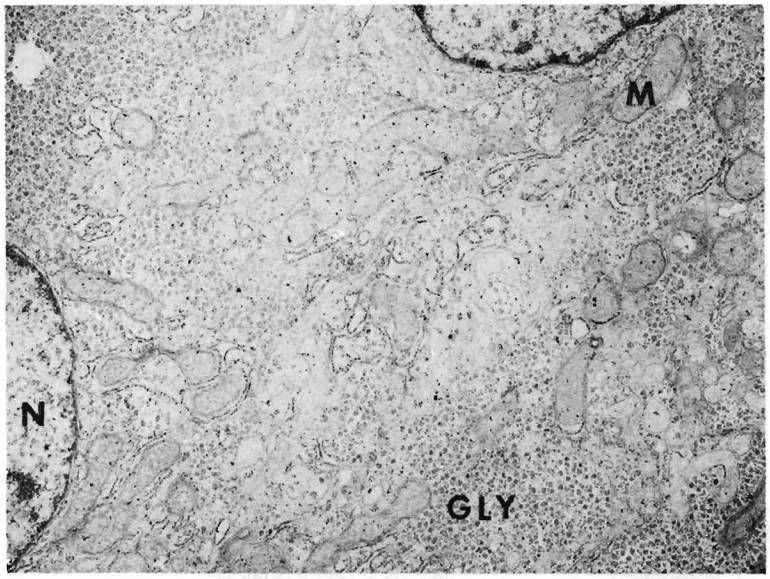

Figure 1.—Micrograph from liver of control channel catfish showing portions of several hepatocytes. M-mitochondria; N-nucleus; Gly-glycogen. 20,000×.

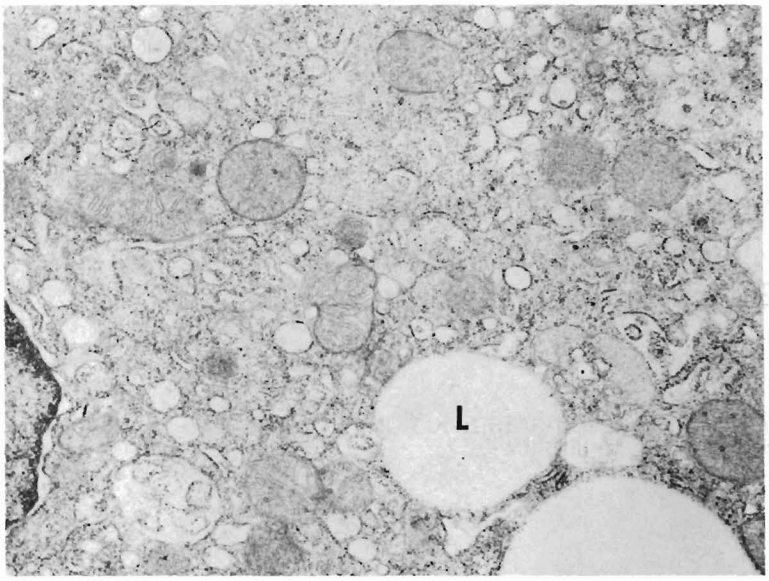

Figure 2.—Portion of hepatocyte from a PCB-treated fish (acute exposure) showing vesicular profiles of smooth and rough endoplasmic reticulum and lipid droplets. L-lipid. 25,000×.

[1]Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

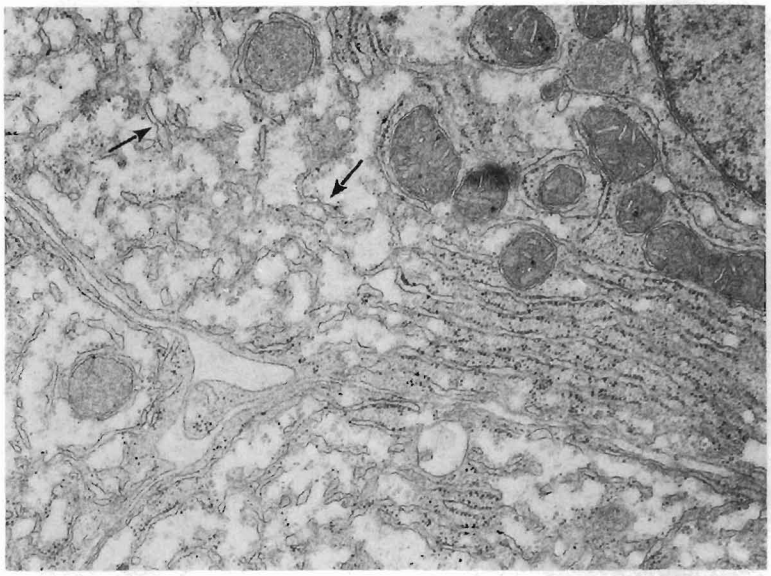

Figure 3.—Hepatocyte, following 21 days exposure to PCB (subacute exposure), showing proliferation of tubular profiles of smooth endoplasmic reticulum (arrows). 25,000 ×.

Tissues for electron microscopy were minced in 4 percent formaldehyde-1 percent glutaraldehyde, in 200 milliosmolar phosphate buffer pH 7.4, and fixed for 2 hours (McDowell and Trump, 1976). Following postfixation in 1 percent OsO_4, tissues were dehydrated in graded ethanol solutions and embedded in Epon (Luft, 1961). Semithin sections of Epon embedded material were stained with toluidine blue and viewed under a light microscope. Thin sections were stained with uranyl acetate and lead citrate, and viewed under an AEI-6B electron microscope.

The mean liver to body weight ratios for control and acute PCB-treated fish were not statistically different. Similarly, microsomal protein values were unchanged. Cytochrome P-450 increased approximately 39 percent in PCB-treated fish when expressed as *n* moles per milligram protein or *n* moles per gram liver. The largest degree of change was seen in the amount of cytochrome b_5 which increased 2.5-fold. The specific activity of NADPH-cyt-c reductase was increased to 1.6 times the control value. Aminopyrine demethylase did not change.

After 21 days of PCB treatment, cytochrome P-450 and b_5 were increased twofold or more over control values. Again, microsomal protein did not change. NADPH-cyt-c reductase values were higher than control but were in the same range of those at 7 days. Aminopyrine demethylase, unchanged during the acute study, increased to 3.4 times the control activity.

Following PCB exposure, toluidine blue-stained thick sections revealed increased numbers of aqua-colored lipid droplets. Hepatocytomegaly was an inconstant finding; however, occasional hepatocytes with enlarged, rounded profiles were observed.

Electron microscopy after acute PCB treatment showed changes in hepatocytes which were primarily restricted to the endoplasmic reticulum and to lipidlike material. Frequently, rough endoplasmic reticulum (RER) was increased. Cisternae were expanded and contained homogeneous material of low to medium electron density. In addition, the endoplasmic reticulum was often seen in circular and vesicular profiles rather than parallel arrays as in controls (Fig. 1, 2). Occasional nuclear alterations, such as large indentations, were noted.

Subacute exposure produced ultrastructural alterations similar to those seen in mammals. Smooth endoplasmic reticulum (SER) was greatly increased over controls, and was seen as circular and tubular profiles as well as lateral outgrowths of RER cisternae (Fig. 3). Long meandering tracks and stacks of SER as well as membrane whorls 5-10 layers thick were evident (Fig. 4, 5). RER was still seen as vesicular profiles and parallel cisternae. "Liposomes" and lipid droplets appeared increased with nuclear atypia still an occasional finding.

Morphologic findings after PCB treatment in fish liver revealed close correlation to the biochemical results. The acute, moderate response of the microsomal MFOS was related to moderate alteration in endoplasmic reticulum morphology. RER was increased, seen as single meandering tracks and circular, vesicular profiles. SER, in scattered foci, appeared to be increased over the scant number of profiles associated with the control livers. The large increases in MFOS activity which characterized the subacute response to PCB were accompanied by extensive changes in endoplasmic reticulum morphology and content. SER was greatly increased, occurring at times as myelinlike membrane whorls.

The findings of this study are promising in that a bioassay based upon biochemical and morphologic studies of pollution-induced cellular alterations in fishes seems possible. Such an assay would contribute much to our understanding of the relationship of water quality and diseases of fish.

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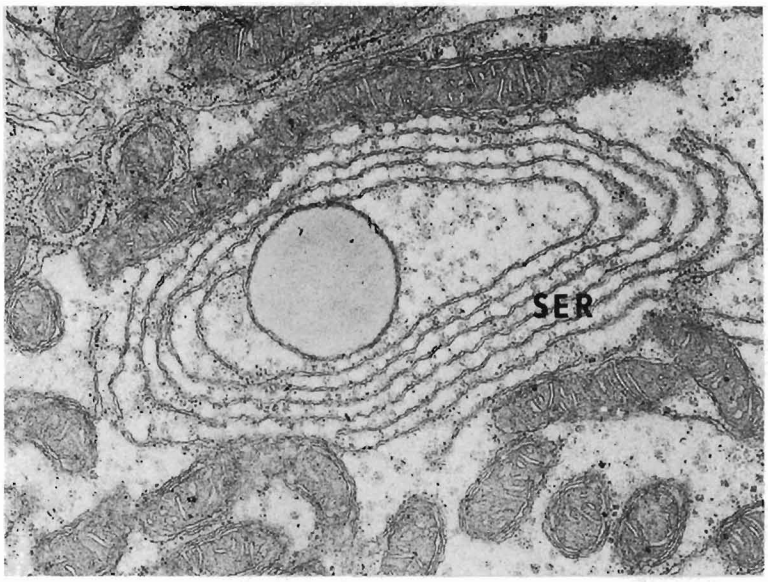

Figure 4.—Small membrane whorl of smooth endoplasmic reticulum (SER) surrounding a lipid droplet following subacute exposure to PCB. 25,000 ×.

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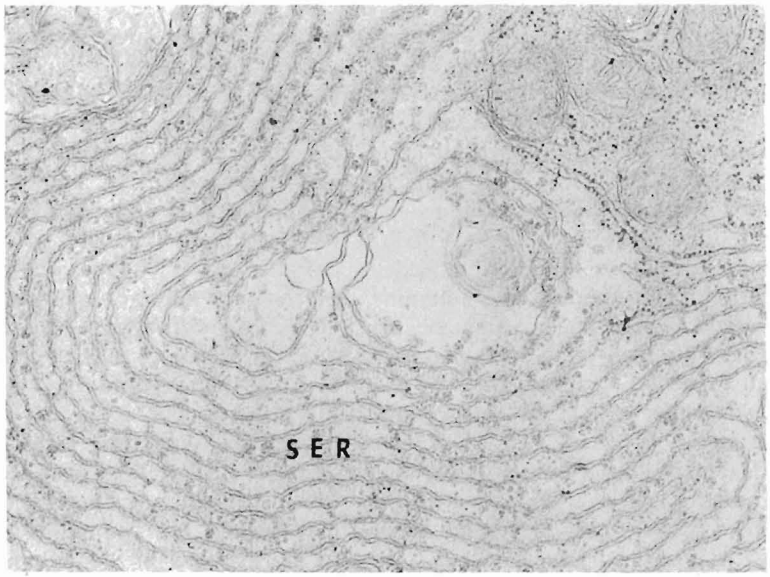

Figure 5.—Portion of large membrane whorl composed of multiple layers of SER separated by glycogen-filled cytoplasm. 20,000 ×.

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