The Effects of a Polychlorinated Biphenyl (Aroclor 1254) on the White Pelican: Ultrastructure of Hepatocytes

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INTRODUCTION

Polychlorinated biphenyls (PCB's) have been commercially manufactured in the United States since 1929 (RISEBROUGH and DE LAPPE 1972). They are not readily biodegradeable and now are present in much of the earth's environment. The long range effect of PCB's on humans and other organisms is not completely understood. The objective of this study was to determine quantitatively the effect of a known dose of PCB's on white pelican (*Pelecanus erythrorhynchos*) hepatocytes. The white pelican was chosen for this study because high levels of PCB's have previously been found in these birds (GREICHUS *et al.* 1973).

MATERIALS AND METHODS

Nestling white pelicans were collected from LaCreek National Wildlife Refuge, Martin, South Dakota, on July 7, 1972, and transferred to cages at the laboratory. Nine experimental birds received 100 mg of PCB's (Aroclor 1254, Monsanto Chemical Co.) per day orally, while nine other birds served as controls. Approximate average weight of the birds was 6.7 kg at the beginning of the experiment (GREICHUS *et al.* 1977). Average death weight was approximately 4.8 kg. After ten weeks, PCB treatment ceased and all the birds were stressed 14 days by decreasing their food intake by one-half. On the 14th day of the stress period, all the birds were killed by intracardiac injection of air.

Immediately after death liver tissue was removed with biopsy needles and placed in a cold (4° C) solution of 5% glutaraldehyde and 0.2% sodium thioglycolate in 0.05 M potassium phosphate buffer (pH 7.2). After 1-2 hours, the samples were rinsed 30 minutes in cold 0.2% sodium thioglycolate in the buffer solution. This rinse was repeated and followed by a 30 minute

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cold rinse in the buffer. The buffer rinse was repeated and the tissues post-fixed in cold 1% osmium tetroxide in 0.05 M potassium phosphate buffer (pH 7.2). The tissue was dehydrated by successive immersions in acetone rinses: 25% (10 min), 50% (30 min), 75% (30 min) and 100% (90 min). The tissue was infiltrated with a mixture of equal volumes of acetone and epoxy resin and embedded in pure epoxy.

Approximately 10 thin sections (50-90 nm) were cut from each liver sample. The sections were stained with 2% uranyl acetate and 0.5% lead citrate then viewed at approximately 2,000 magnifications using an RCA EMU-3G electron microscope. One section from each bird was used for electron micrographs. At least 15 micrographs were taken from each individual sample.

Quantitative Procedures

Quantitative information was obtained from ten different hepatocytes from each sample by the following procedures: 1. Hepatocyte size was calculated by measuring length and width of those hepatocytes in which nuclei and accompanying nucleoli were visible. Since nuclei are usually centrally located within hepatocytes, such sections were assumed to provide measurements taken through the approximate center of hepatocytes. Area was calculated as product of length times width (Table 1). 2. Nuclear and nucleolar size was determined in each of the 10 hepatocytes by measuring length and width. Area was calculated as the product of length times width (Table 1). 3. Visible mitochondria in each of the 10 hepatocytes were counted and the numbers averaged (Table 1). 4. Visible cristae were counted and the numbers averaged in 16 randomly chosen mitochondria in each of the 10 hepatocytes (Table 1). 5. Vacuoles (lysosomes, microbodies, or other membrane-bound vacuoles) were counted in each of the 10 hepatocytes and the numbers averaged (Table 1). 6. Mitochondrial size was determined by measuring length and width of 16 randomly chosen mitochondria in each of the 10 hepatocytes. Area was calculated as the product of length times width (Table 1). 7. Analysis of variance was used to determine if significant differences existed in the above parameters of the PCB treated and control pelican hepatocytes.

RESULTS AND DISCUSSION

The principal ultrastructural features of the normal pelican liver (Fig. 1) closely resembles those described for the Japanese quail (ATWAL 1973). The data derived from examining 90 hepatocytes from

control pelicans and 80 hepatocytes from PCB treated pelicans is given in Table 1.

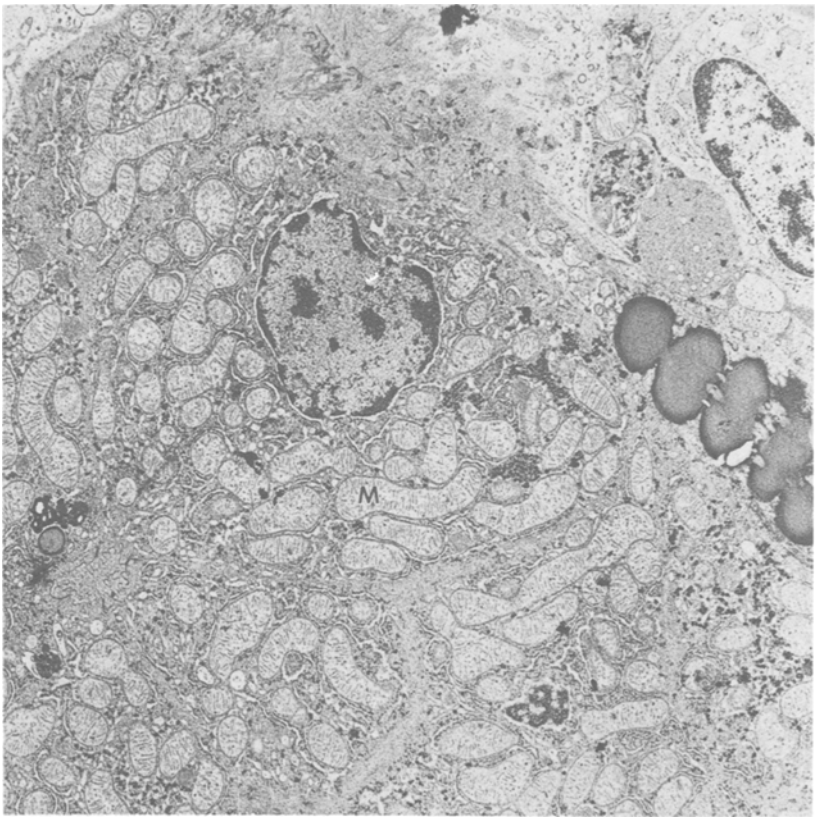

Figure 1. Electron micrograph of liver from control pelican. Note normal appearing mitochondria (M). Approximate magnification - 9,000 X.

Comparison of the hepatocyte ultrastructural morphologic characteristics indicated a 22% greater hepatocyte size in the treated than in the control pelicans. Although analysis of variance did not show statistical significance at the $P < 0.05$ level, this agrees with results of previous work in which livers from PCB-treated pelicans weighed 15% more than those of control birds (GREICHUS *et al.* 1975). The increase in liver size is due to hypertrophic rather than hyperplastic change. It has been reported that PCB-treated rats had hepatic hypertrophy and greater liver weights than similar untreated rats (KIMBROUGH *et al.* 1972).

TABLE 1

Comparison of Size and Morphologic Characteristics of Hepatocytes of Pelicans Treated With PCB and Untreated Controls

Discription	Control	Treated	% Difference between groups	Standard Deviations Control	Standard Deviations Treated	Values of F
Cross-section area of hepatocytes	876.0 um^2*	1069.0 um^2*	22	340.43	383.83	1.96
Cross-section area of nucleus	161.0 um^2*	169.0 um^2*	5	65.54	61.38	0.02
Cross-section area of nucleolus	7.1 um^2*	6.5 um^2*	8	6.02	6.47	0.62
Average number of mitochondria per cross-section of hepatocyte	43.0	54.0	25	15.12	14.34	12.07**
Size of mitochondria	5.9 um^2*	5.6 um^2*	5	1.50	1.24	0.60
Number of cristae per mitochondrion	10.3	8.2	20	2.20	1.82	8.39***
Number of vacuoles per cross-section of hepatocyte	11.5	14.1	22	5.13	6.06	3.09

* Area calculated as product of length times width.
** Treated group significantly different from controls ($P < 0.05$).
*** Treated group significantly different from controls ($P < 0.01$).

Hepatocytes from treated pelicans had significantly greater numbers of mitochondria (25%) than did those of control birds ($P < 0.01$). The mitochondria from the treated group were slightly smaller than those of the controls and appeared rounded and swollen instead of long and slender (Fig. 2). These difference in mitochondrial characteristics are similar to those described by VON ERICH RUTSCHE and BROZIO (1975). Other studies involving DDT and dieldrin treated rats revealed atypical mitochondria, confluence of two mitochondria within a double outer membrane, or loss of a portion of the outer membrane (KIMBROUGH *et al.* 1972). In the present study, only once were two mitochondria observed within a single membrane.

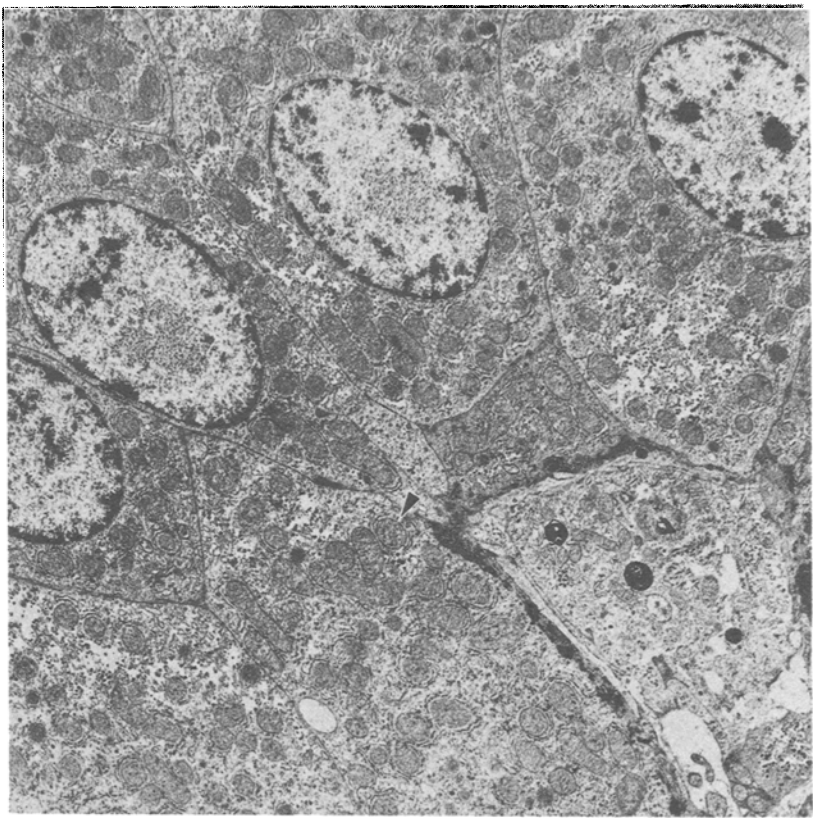

Figure 2. Electron micrograph of liver from PCB-treated pelican. Note rounded mitochondria (arrow). Approximate magnification-8,500 X.

There were significantly fewer cristae ($P < 0.05$) per mitochondria in the treated bird hepatocytes as compared to those of the controls. Changes similar to these have been described previously (VON ERICH RUTSCHKE and BROZIO 1975). KIMBROUGH *et al.* (1971) reported a greater number of cristae in hepatic mitochondria of rats treated with dieldrin, or a combination of dieldrin and DDT as compared with hepatic mitochondria in control of rat livers. Occasionally the cytoplasm of hepatocytes in PCB-treated pelicans appeared electron-lucent. This agrees with the report of KIMBROUGH *et al.* (1971).

The number of vacuoles (lysosomes, microbodies, or other membrane-bounded vacuoles) was 22% greater in the treated group than in controls. This difference was not significant at the $P < 0.05$ level. Some workers have reported more cytoplasmic vacuoles and fat deposits in hepatocytes of various species treated with chlorinated hydrocarbons, kepone, polychlorinated triphenyls, or PCB's (ALLAN and ABRAHAMSON 1972, WRIGHT *et al.* 1972, BRUCKNER *et al.* 1973), no difference in amount or arrangement of hepatic fat deposit was found between PCB treated and control pelicans in this study.

There were no noticeable differences in hepatic collagen or smooth endoplasmic reticulum between the two groups. Greater than normal amounts of collagen and smooth endoplasmic reticulum have been found in livers of rats and guinea pigs treated with PCB's (KIMBROUGH *et al.* 1972, AMAGASE 1975). The glycogen content of the hepatocytes varied to such an extent that it was not possible to detect differences between the two groups.

SUMMARY

White pelicans were each given 100 mg of polychlorinated biphenyls (Aroclor 1254) per day for 10 weeks. After the treatment period, the bird's food supply was decreased 50% for 2 weeks. Measurements taken from transmission electron micrographs of the pelican's liver tissue revealed hepatocytes of the treated birds averaged 22% larger in cross-section area than those of controls. The number of vacuoles (lysosomes, microbodies) per hepatocyte was 22% greater. the number of mitochondria per hepatocyte was 25% greater while 25% fewer cristae per mitochondrion were present in treated as compared to control birds.

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