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Changes in Biliary Proteins in Rats fed on diet containing PCB.

Kazuhiko SUZUKI

Polychlorinated biphenyls (PCB) feeding, stimulates proliferation of smooth surfaced endoplasmic reticulum (SER), slightly increases in secondary lysosomes near bile canaliculi and varieties of myelin-like figures in hepatocytes. No differences were observed in the contents of biliary protein and cholesterol in PCB-treated rats and those of controls. But the concentration of phospholipid in bile of PCB-treated rats was significantly lower than that of controls. Electrophoretic analyses by SDS-polyacrylamide gels showed twelve bands of polypeptides in biles of both groups, which indicated no major qualitative changes between PCB-treated and control rats. The biliary protein of PCB-treated rats was higher in polypeptides with smaller molecular weights than that of controls.

KEY WORDS : PCB, Secondary lysosomes, Biliary protein, Biliary phospholipid.

In rats, diet containing 0.1% of Polychlorinated biphenyls (PCB) has caused the accumulation of cholesterol and triglyceride in the liver (1,2). Ishikawa *et al.* (3) reported that HDL-cholesterol was increased in rats received PCB. The PCB-treated rats showed proliferation of smooth surfaced endoplasmic reticulum (SER) (4) and increase in cholesterol in serum (1,2). In the present work we studied the effects of PCB on the contents of cholesterol, phospholipid, protein and composition of protein in bile of rats, where their cholesterol in serum and liver were high.

MATERIALS and METHODS

Male Wistar strain rats (Tokushima Jikken-Dobutsu Kenkyusho, Tokushima, Japan), weighing about 80 g, were given diet containing 30 % casein as described by Kato and Yoshida (1). The experimental rats were fed the diet containing 0.1 % of PCB *ad libitum* for 3 weeks. They were anesthetized by the intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) after 15 h starvation. Bile ducts were cannulated with PE-10 tubings and rats were placed in constant temperature (30℃) during collections of bile. To avoide mixing with pancreatic secretion careful ligations of bile ducts were performed. Bile were collected for 3 h after cannulation then kept at 0℃. Blood samples were obtained from *vena cava inferior*. Liver tissues for electron microscopy were

promptly fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h and postfixed in 1 % osmium tetroxide in 0.2 M cacodylate Epon 812 and sectioned with LKB ultramicrotome (LKB, Co., Ltd., Stockholm, Sweden). They were stained with uranyl acetate and lead citrate. The sections were examined in Hitachi HS-7D elctron microscope (Hitachi, Co., Ltd., Tokyo, Japan). Cholesterol in serum and bile was determined by the method of Zak (5). Total lipid in bile was extracted by the method of Folch *et al.* (6). Phospholipid in bile was measured as inorganic phosphate after oxidation of total lipid with 60 % $HClO_4$ by the method of Fiske-Subbarrow (7). Biliary protein was determined by the method of Lowry *et al.* (8). Biliary protein was separated by 10 % polyacrylamide disc electrophoresis in 0.1 % SDS, and stained with Coomassie brilliant blue as described by Weber and Obsorn (9). The stained bands were scanned at 550 nm with a Shimazu Dual Wavelength Chromato Scanner Cs 900 (Shimazu, Co., Ltd., Kyoto, Japan). The ratios of weights of subfructions were calculated from the areas of the scanned peaks with an Apple Graphics Tablet (Apple computer Inc., California, USA) (10).

RESULTS

Macroscopic observations

The final body weights of PCB-treated rats were lower than those of the controls. The relative weight of the liver were significantly larger in PCB-treated animals. The relative weights of thymus and spleen were smaller in PCB-treated group than those of conrols *(Table 1)*.

Table 1. Body and organ weights of rats fed on diets containing 0.1 % PCB and control

		PCB	Control
Body weight	(g)	165.7 ± 29.4*	228.6 ± 6.3
Liver	(%)	7.13 ± 0.69*	4.30 ± 2.72
Spleen	(%)	0.21 ± 0.03*	0.25 ± 0.02
Thymus	(%)	0.18 ± 0.03*	0.33 ± 0.05

Values are means±SD of 6 (PCB group) or 5 (control group) rats.
Organ weights are expressed as g/100 g body weight. * $p<0.05$.

Electron microscopic observations

PCB-treated rats, showed marked proliferation of SER and vesicular formation in the hepatocytes. Autophagic vacuoles containing heterogeneous, dense materials and smooth membranes were cleary seen near bile canaliculi. Dense bodies and secondary lysosomes were increased in pericanalicular areas in the cells *(Fig. 1)*. Many myelinlike figures were

present in continuity with proliferated SER *(Fig. 2)*. Mitochondria in general appeared normal but some mitochondria were markedly irregular in shape.

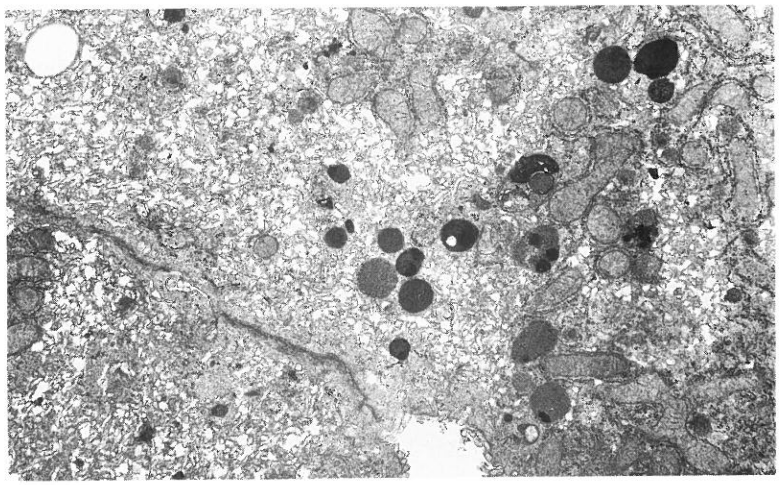

Figure 1. Electron micrographs of rat liver fed on diet containing PCB. Note marked increase in SER in the pericanalicular area. Autophagic vacuoles are seen. × 10000.

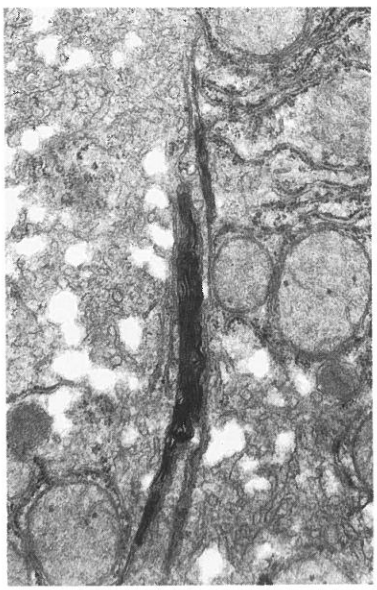

Figure 2. Electron micrographs of rat liver fed on diet containing PCB. Note myelen-like figures continuous with proliferated SER. SER shows vesicular formation. × 26000.

Biliary protein, cholesterol and phospholipid

The contents of protein and cholesterol in bile of PCB-treated rats were similar to those of controls *(Table 2)*. Phospholipid content in bile of PCB-treated rats was lower than those of controls. In contrast, the concentration of serum cholesterol in PCB-treated rats was significantly higher than that of controls.

Table 2. The concentration of lipid and protein in bile, and cholesterol in serum of PCB-treated and control rats

	PCB	Control
Cholesterol in bile (*mg/100ml*)	49.4 ± 9.9	47.9 ± 4.7
Phospholipid in bile (*μmole/100ml*)	203.6 ± 95.0 *	338.5 ± 52.1
Protein in bile (*mg/ml*)	3.87 ± 0.91	2.95 ± 0.73
Cholesterol in serum (*mg/100ml*)	185.2 ± 40.7 *	89.3 ± 8.5

Values are means±SD. * $p<0.05$.

The composition of biliary protein

Twelve bands of proteins were seen in bile of the rats treated with PCB and controls *(Fig. 3)*. The bands 12, 10, 8 and 7 in PCB-treated rats were higher than those of controls. Particulary the band 12 in PCB-treated rats was markedly increased. The amount of small polypeptides, smaller than band 9, relative to the amount of larger polypeptides in bile of PCB-treated rats *(Fig. 4)* was higher than that of controls (0.459±0.122 in PCB-treated rats, 0.266±0.099 in controls; mean±SD).

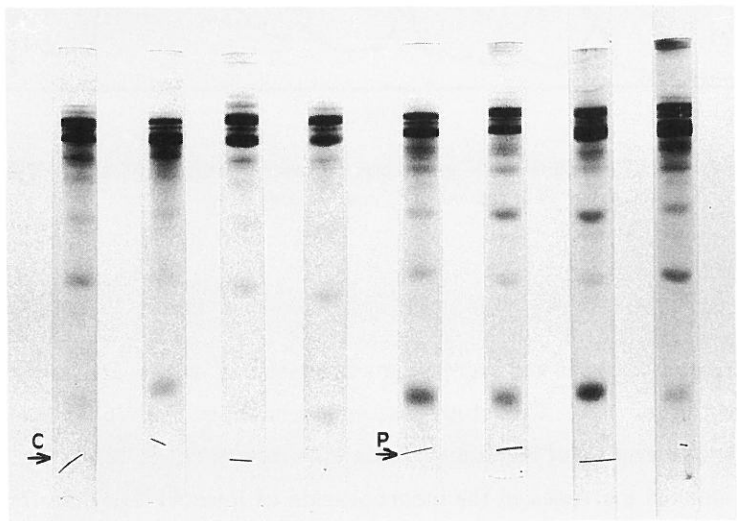

Figure 3. SDS-polyacrylamide gel electrophoretic separation of the protein of biles from controls (C) and PCB-treated rats (P). Fifty *μg* of protein was applied to each lane. Arrow shows gel front.

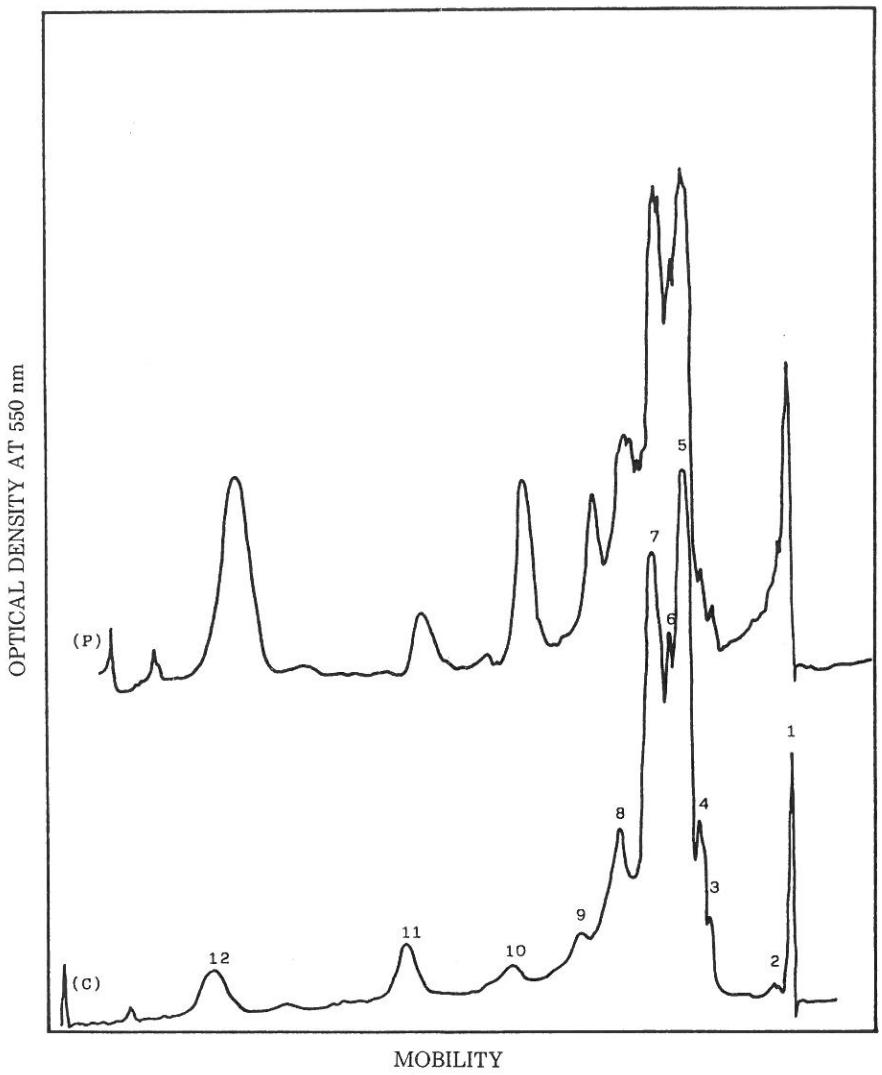

Figure 4. SDS-polyacrylamide gel electrophoretic separation of the polypeptides of biles from PCB-fed rats (P) and controls (C).

DISCUSSION

The PCB-treated rats showed increase in cholesterol in serum (2) and proliferation of SER in the hepatocytes (4). This study also has comfirmed these phenomena. Proliferation of SER caused elevation of hepatic microsomal enzymes (1, 11, 12). Kato and Yoshida (13) indicated marked increases in the incorporation of injected $^{3}H_2O$ into hepatic cholesterol and in the activity of liver microsomal 3-hydroxy-3-methylglutaryl Coenzyme A reductase in rat liver treated with PCB. They also indicated that the contents of cholesterol in liver and high density lipoprotein (HDL) in serum of PCB-treated rat were higher than

those of controls. Cholesterol accumulated in the hepatocyte might move either to blood or to the bile. But cholesterol content in bile of PCB-treated rats were similar to those of controls, in contrast to their high concentration of cholesterol in serum.

Osmiophilic particles in secondary lysosomes in Figure 1, might be lipoproteins (14). The source of the lipoproteins in the lysosomes might be serum lipoproteins and membranes of proliferated SER injured by lipid peroxide or PCB. Although the lipofuscin-like bodies or autophagic vacuoles containing heterogeneous, dense materials were increased in number near a bile canaliculus, the phospholipid in bile of PCB-treated rats was lower than that of controls. Hruban *et al.* (15) indicated that abnormalities either in protein synthesis or in cholesterol synthesis might produce myelin-like structures. Numerous secondary lysozomes near bile canaliculus may have less contribution to the secretion process of phospholipid to bile. Physiologically, low level of phospholipid in bile may partly inhibit the absorption of PCB and fat soluble vitamins (16) from gut.

Lysosomes promote the overall process of intracellular protein degradation. de Duve and Wattiaux suggested that lysosomes discharge their cnotents at the bile canalicular face of the hepatocyte (17). Numerous secondary lysozomes near bile canaliculus discussed above may be associated with the secretion of biliary protein. In fact, there were increase in proteins of lower molecular weight.

Mullock *et al.* (18) have distinguished sixteen bile proteins with two dimensional agarose-polyacrylamide gel electrophoresis. They showed that bile contained significant amount of serum protein such as albumin, IgA, and IgG. Swell *et al.* (17) reported that human bile contained apolipoproteins A-I, A-II, C-II, C-III, and B. Apo-A-I and A-II appeared in the bile when rat liver was perfused with the perfusate added human HDL. High level of HDL-cholesterol may be responsible for secretion of smaller polypeptide of bile of PCB-treated rats.

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