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TRANSPORT AND CELLULAR UPTAKE OF POLYCHLORINATED BIPHENYLS (PCBs)—II

CHANGES *IN VIVO* IN PLASMA LIPOPROTEINS AND PROTEINS OF PIGEONS IN RESPONSE TO PCBs, AND A PROPOSED MODEL FOR THE TRANSPORT AND CELLULAR UPTAKE OF PCBs

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Abstract—The complex distribution of polychlorinated biphenyl (PCB) isomers and congeners amongst plasma fractions of the pigeon suggests that the lipid and apolipoprotein components of lipoproteins, as well as plasma proteins, may be important in transporting PCBs to tissues (Borlakoglu *et al.*, *Biochem. Pharmac.* **40**, 265 (1990)). Pigeons were injected with the commercial PCB mixture Aroclor 1254 (1.5 mmol/kg body weight). After 120 hr triacylglycerol-like droplets accumulated in hepatocytes ('fatty liver syndrome'), there was proliferation of the hepatic smooth endoplasmic reticulum, and plasma concentrations of triacylglycerol and total cholesterol increased. This was accompanied by significant decreases in plasma concentrations of total protein, total apolipoproteins of the low density lipoprotein (LDL) and the high density lipoprotein (HDL) fractions, and albumin and by a significant increase in that of urea, indicating increased protein breakdown. These results suggest that Aroclor 1254 increased hepatic lipid synthesis, but decreased hepatic production of albumin and apolipoproteins. This would explain the accumulation of triacylglycerol in the liver and the increase in the proportion of triacylglycerol to apolipoprotein in the total lipoproteins. From the evidence presented, a model is proposed based on the association of PCBs with hydrophobic domains of lipids and proteins for the transport of PCBs by plasma fractions, their uptake into cells and intracellular metabolism, and their accumulation in adipose tissue.

The preceding paper [1] describes the complex but distinctive distribution of PCB§ isomers and congeners amongst plasma lipoproteins and the lipoprotein-poor protein fraction 24 hr after pigeons had been injected with [^{14}C]4-monochlorobiphenyl or [^{14}C]2,2′,5,5′-tetrachlorobiphenyl, and 120 hr after pigeons had been injected with the commercial PCB mixture Aroclor 1254 which contains about 80 PCB isomers and congeners. The results obtained suggested that lipoproteins and plasma proteins are important in the transport of PCBs in plasma and hence, by implication, in the uptake of PCBs into tissues.

The accumulation of lipid droplets in the cytosol of hepatocytes in response to treatment with PCBs is a well known feature, albeit the mechanisms are not understood. It is not known whether this accumulation is the result of an adaption of hepatocytes to PCBs, or whether it is the result of a disturbance of lipoprotein lipid transport and, perhaps, lipid metabolism. It is probable that the enhanced formation of lipid droplets in the cytosol of hepatocytes allows PCBs to be sequestered and stored in lipid droplets. This scavenging of PCBs could prevent cellular toxity such as PCB-adduct formation with cellular proteins or nucleotides, since metabolic activation of isomers and congeners by cytochrome P450-dependent monooxygenases is prevented. The sequestration of PCBs might reduce the formation of PCB electrophiles by their metabolic activation that could cause detrimental events in the cell and lead to necrosis and cell death (e.g. 'suicidal' activation), due to enhanced oxidative stress.

By contrast, the accumulation of lipid droplets could also be the result of severe disturbance of lipid transport and metabolism. To address this question experimentally we have investigated the effects *in vivo* of Aroclor 1254 on concentrations in pigeon plasma of total apolipoprotein, triacylglycerol and total cholesterol of lipoproteins, and on the lipoprotein-poor protein fraction, and discuss whether these effects can help to explain the accumulation of lipids in hepatocytes ('fatty liver syndrome').

The results obtained have led us to propose a model for the transport of PCBs in blood and their uptake by tissues. It is hoped that this will increase our understanding of the response of organisms to PCBs, which are one of the most abundant pollutants, in the environment.

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§ Abbreviations used: PCB, polychlorinated biphenyl; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoproteins; HDL, high density lipoprotein.

MATERIALS AND METHODS

Materials and animals

These are described in the preceding paper [1]. In addition, Chrompak CP Sil 88 was obtained from Packard Instruments (Pangbourne, U.K.) and standard mixtures of methyl esters of fatty acids from Sigma Chemical Co. (Poole, U.K.) and from Supelco (Sawbridgeworth, U.K.). The IUPAC system of numbering individual PCB isomers and congeners (see Ref. 2) has been used where convenient.

Methods

Pigeons of 300 ± 15 g body weight (mean value ± SD; N = 23) were given a single intraperitoneal injection (up to 1 mL total volume) of 500 mg (1.5 mmol assuming an average M_r of 326) of Aroclor 1254 in corn oil per kg body weight at 10 a.m. and killed 24, 48, 68 and 120 hr later. Control pigeons (N = 9) were given a single intraperitoneal injection of 1 mL of corn oil at 10 a.m. and killed 24 or 120 hr later.

Plasma was prepared by centrifugation of samples (10 mL) of blood in plastic tubes that contained approx. 0.3 mL heparin (5000 units/mL) for 15 min at 500 g_{av} at room temperature. Portions of plasma (4 mL) were stored at −20° and thawed once. The concentrations of triacylglycerol and of total cholesterol in samples of plasma (or occasionally of serum) were measured using 'Merkotest Triglycerides, enzymatic' and 'MERCK System Cholesterol Enzyme Mixture' kits obtained from BDH (Poole, U.K.) and the 'Cholesterol Calibrate' kit from the Sigma Chemical Co. A 'KONE' analyser (KONE Laboratory Medics Ltd, Stockport, U.K.) was used to measure plasma concentrations of total protein with the 'Total Serum Protein' assay kit from BCL (Lewes, U.K.), albumin with the 'SpecTru^R' assay kit from Lancer^R, County Kildare, Ireland, and urea with the 'Dri-Stat^{TM}' assay kit from Beckman Instruments (U.K.) Ltd (High Wycombe, U.K.). Assays were in duplicate, with appropriate controls.

Preparation and chemical analysis of plasma fractions. The portomicron plus VLDL, HDL, LDL and lipoprotein-poor protein fractions were prepared by ultracentrifugation as described in the preceding paper [1]. The concentrations of triacylglycerol and of total cholesterol in samples of the lipoprotein fractions were measured as described above for plasma. In addition, the total apolipoprotein concentration was measured using the 'Pierce Coomassie Protein Assay' kit supplied by Pierce (U.K.) Ltd (Cambridge, U.K.), and by the method of Lowry *et al.* [3]. The concentration of protein in the lipoprotein-poor protein fraction was measured using the 'Total Serum Protein' assay kit from BCL. Portions of the LDL and HDL fractions were stained with Sudan Black and their purity checked by PAGE, as described in Ref. 1.

To enable direct comparisons to be made, changes in the concentrations of lipids and proteins in response to Aroclor 1254 are expressed per mL of plasma.

Fatty acid composition of lipids accumulating in liver. The liver was removed 120 hr after pigeons had been injected with Aroclor 1254, washed free from superficial blood with ice-cold 0.15 M KCl and finely chopped. Portions (2 g wet weight) were homogenized using a Potter-Elvejhem homogenizer at room temperature with 24 mL of chloroform–methanol (2:1 v/v) and the chloroform phase retained. Further portions of liver were homogenized in this way at 4° for 2 min with 0.15 M KCl (3 mL/g wet weight liver), the homogenate centrifuged at 10,000 g_{av} for 30 min at 4°, the floating fat layer removed, extracted with chloroform-methanol (2:1 v/v) at room temperature and the chloroform phase retained. Both sets of chloroform extracts were washed with 0.8% (w/v) KCl, dried over anhydrous sodium sulphate, evaporated to dryness, $C_{21:0}$ methyl ester added as internal standard, and the mixture redissolved in 2:1 (v/v) chloroform–methanol. Portions of this extract were taken to dryness, and fatty acid methyl esters prepared by heating the dried extract in a sealed ampoule under N_2 with 1 mL of 11.6 M HCl/methanol (0.1:1 v/v) for 30 min at 100°. The methyl esters were dissolved in *n*-hexane and 1-μL portions separated by capillary GC using a column (50 m length and 0.22 mm i.d.) of Chrompak CP Sil 88 attached to a Packard 436 gas chromatograph fitted with a flame ionization detector. The flow rate of H_2 as carrier gas was 0.84 mL/min, with a splitter ratio of 55:1. Hydrogen and N_2 were supplied to the detector at 30 mL/min, with air at about 220 mL/min. The injector and detector were at 270°. The temperature was programmed at 190° for 15 min, raised at 30°/min to 270° and maintained at 270° for 7 min. The instrument was inferfaced with a Digital Micro PDP 11/23 computer with 'Multichrom' software to quantitate peaks by comparison with the $C_{21:0}$ methyl ester internal standard. A minimum of 230 pmol of $C_{18:2}$ (9, 12) methyl ester could be measured. Methyl esters of fatty acids were identified by comparison with authentic standards.

Light microscopy. Small pieces of liver were fixed either in buffered formalin, Carnoy's or Bouin's solution, and processed as described in Ref. 4, washed repeatedly in 50% ethanol to remove picric acid and stored in 70% ethanol. The material was embedded in paraffin and cut into 5 μm sections. Sections were stained with haematoxylin and eosin, with Sudan Black or Sudan Orange III for lipids, and with periodic acid-Schiff's reagent (PAS) and PAS digest (e.g. α-amylase) for hepatic glycogen content.

Electron microscopy. Small pieces of liver were fixed in 3% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, washed every hour for 5 hr with this solution, and stored at 4° in 0.1 M sucrose/0.1 M sodium cacodylate buffer, pH 7.2. The samples were immersed in 1% (w/v) osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.2, dehydrated in ethanol, embedded in resin, sectioned to 0.1 μm, and stained with uranyl acetate and lead citrate. Sections were examined using a Joel 100S transmission electron microscope at an accelerating voltage of 80 kV [4].

RESULTS

Effects of Aroclor 1254 on plasma and liver lipids

Figure 1 shows the plasma concentrations of triacylglycerol and total cholesterol in response to treatment with Aroclor 1254. No significant changes from

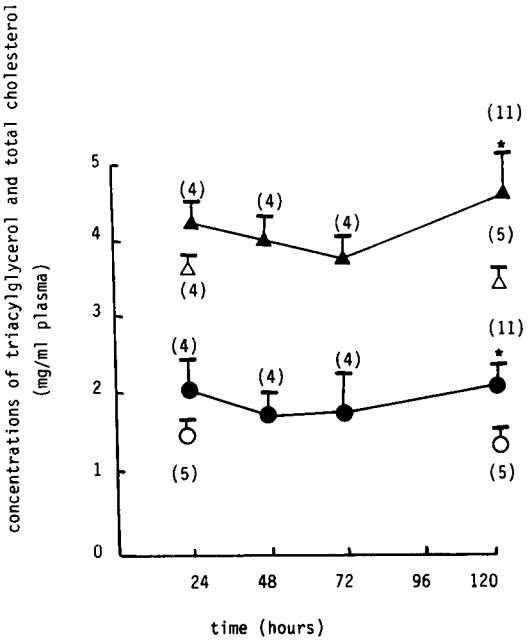

Fig. 1. Plasma concentrations of triacylglycerol and total cholesterol after injecting pigeons with Aroclor 1254. Pigeons were injected with 1.5 mmol of the commercial PCB mixture Aroclor 1254 or with corn oil and killed at the times shown. Details of the preparation of plasma and measurements of triacylglycerol and total cholesterol are given in Materials and Methods. Plasma triacylglycerol in treated (●) and in control (○) pigeons; plasma total cholesterol in treated (▲) and in control (△) pigeons. Values are means ± SD, with the numbers of independent experiments shown in parentheses. * $P < 0.05$ compared to mean values of control at 24 and 120 hr.

control values occurred during the first 68 hr, but both concentrations showed small but significant ($P < 0.05$) increases after 120 hr compared to the average value of the 24 and 120 hr controls.

This delayed response is in striking contrast to the effects of Aroclor 1254 observed in the liver. When sections of liver from pigeons treated with Aroclor 1254 for 120 hr were examined by light microscopy, vacuoles were apparent in the hepatocytes as judged by staining with haematoxylin and eosin. Staining with Sudan Black and Sudan Orange III showed that these vacuoles were large lipid droplets, and densely packed smaller lipid droplets were frequently observed in the cytoplasm of these hepatocytes, though there was considerable variation between pigeons in this response to Aroclor 1254. When sections of liver were examined by electron microscopy, a time-dependent accumulation of lipid was observed. Small, finely-dispersed lipid droplets were already present 24 hr after treatment with Aroclor 1254. These increased in size and number 48 and 68 hr after treatment, and by 120 hr closely packed large lipid droplets were present which almost filled the cytoplasm of the cells. This accumulation of lipid ('fatty liver syndrome') was not observed with the control pigeons that had been injected with corn oil and thus no comparison could be made to controls. Analysis of the fatty acid composition of the lipid droplets that accumulated 120 hr after treatment (Table 1) showed an extremely low proportion (4.7 mol %) of polyunsaturated fatty acids. This resembles the composition of the triacylglycerols of adipose tissues, and is strikingly different from the high proportion of polyunsaturated fatty acids present in the whole liver of control (33.5 mol %) and Aroclor-treated pigeons (27.6 mol %), which probably reflects the fatty acid composition of hepatic membrane phospholipids.

Examination by electron microscopy showed that this accumulation of triacylglycerol-like lipid droplets in the liver 120 hr after treatment with Aroclor 1254 was accompanied by substantial proliferation of the membranes of the smooth endoplasmic reticulum [4]. There was no evidence of proliferation of the rough endoplasmic reticulum. This overall proliferation of endoplasmic reticulum membrane was confirmed by measuring the total microsomal protein of the livers of the treated pigeons which showed, on average, a 2.5-fold increase compared to controls.

For comparison, the treatment with Aroclor 1254 resulted in very significant increase in the concentrations of cytochrome P450 (11.4-fold) and cytochrome b_5 (7.7-fold) and in the activities of NADPH-cytochrome *c*-(P450) reductase (5.2-fold), aldrin epoxidase (12.0-fold), ethoxycoumarin *O*-deethylase (9.3-fold) and ethoxyresorufin *O*-deethylase (48.5-fold). There was also a significant but smaller increase in the activity of dimethylnitrosamine *N*-demethylase (2.2-fold), but not in lauric acid 12-hydroxylase. When the cytochrome P450 content of the liver of these treated birds was measured immunochemically using monoclonal antibodies raised to forms of cytochrome P450, increases of up to 100-fold immunoreactive protein could be determined [4]. By contrast the accumulation of lipid droplets in the liver suggested that individual isomers and congeners of the Aroclor 1254 mixture might have inhibited the transport of lipids from the liver as plasma lipoproteins. This was investigated by measuring changes in the concentrations of the triacylglycerol and the total cholesterol components of the portomicron plus VLDL, LDL and HDL fractions prepared from plasma. The plasma concentration of LDL triacylglycerol decreased ($P < 0.05$) between 24 and 120 hr after treatment, and that of cholesterol showed a transient increase 24 hr after treatment (Fig. 2b). Firm conclusions could not be drawn about the effects of Aroclor 1254 on plasma concentrations of the triacylglycerol and total cholesterol components of the portomicron plus VLDL and the HDL fractions due to the variations in the values obtained (Fig. 2a and c) and the low recoveries of these components (63–76% and 74–88%, respectively) compared to their values in plasma. Nevertheless, at 120 hr after treatment there were positive correlations between plasma concentrations of the triacylglycerol and total cholesterol components of the portomicron plus VLDL fraction (Fig. 3a), and between the plasma concentrations of these components of the LDL fraction (Fig. 3b). This suggests that Aroclor 1254 had similar effects on cholesterol and triacylglycerol of the portomicron plus VLDL and LDL fractions.

Table 1. Fatty acid composition of lipid droplets in hepatocytes and of whole liver 120 hr after injecting pigeons with Aroclor 1254

Fatty acid	Fatty acid composition (mol %)		
	Whole liver		Droplets in livers of treated pigeons (5)
	Controls (3)	Treated (4)	
16:0	17.1 ± 0.5	15.9 ± 1.8	18.9 ± 2.1
16:1 (9)	2.3 ± 0.4	1.9 ± 1.6	3.1 ± 0.8
18:0	24.3 ± 0.7	25.1 ± 5.6	27.3 ± 4.4
18:1 (9)	19.5 ± 3.1	26.3 ± 14.8	42.9 ± 4.8
18:1 (11)	3.3 ± 0.2	3.1 ± 1.3	3.6 ± 0.1
18:2 (9,12)	21.5 ± 2.4	17.5 ± 7.2	4.7 ± 2.8
18:3 (6,9,12)	0.14 ± 0.1	0.02 ± 0.03	—
18:3 (9,12,15)	0.23 ± 0.03	0.15 ± 0.04	—
20:3 (8,11,14)	1.7 ± 1.5	0.71 ± 0.69	—
20:4 (5,8,11,14)	7.7 ± 0.8	7.1 ± 5.2	—
20:5 (5,8,11,14,17)	0.30 ± 0.09	0.11 ± 0.04	—
22:4 (4,7,10,13)	0.74 ± 0.21	0.49 ± 0.27	—
22:5 (4,7,10,13,16)	0.38 ± 0.33	0.32 ± 0.19	—
22:6 (4,7,10,13,16,19)	0.83 ± 0.34	1.21 ± 0.40	—

Pigeons were injected with 1.5 mmol of the commercial PBC mixture Aroclor 1254 or with corn oil and killed 120 hr later. Details of the separation of lipid droplets from liver homogenates, the extraction of lipids from these droplets and from whole liver, and analysis of fatty acids of the lipids are given in Materials and Methods. Lipid droplets could not be isolated from control animals and thus no comparison could be made with controls. Values are means ± SD, with the number of independent experiments given in parentheses.

—, not detected.

Effects of Aroclor 1254 *on total plasma protein, total apolipoproteins, albumin and plasma urea*

These changes in plasma and liver lipids in response to Aroclor 1254 led us to investigate whether this treatment led to decreased concentrations of proteins in the plasma.

Figure 4 shows very significant decreases in plasma concentrations of total protein ($P < 0.001$), total apolipoproteins ($P < 0.01$) and albumin ($P < 0.001$) 120 hr after treating pigeons with Aroclor 1254. The decrease in total apolipoproteins had already become significant ($P < 0.05$) 48 and 72 hr after treatment.

The effects of Aroclor 1254 on plasma concentrations of total apolipoproteins of the individual fractions was then measured (Fig. 5). The concentration of the total apolipoproteins of the portomicron plus VLDL fraction (Fig. 5a) was significantly higher ($P < 0.05$) 24 and 48 hr after treatment and then declined towards the control values. Values for the total apolipoproteins of the LDL fraction remained significantly depressed ($P < 0.001$) between 24 and 120 hr after treatment (Fig. 5b), and values for the total apolipoproteins of the HDL fraction were also depressed ($P < 0.001$) 120 hr after treatment.

Finally, Fig. 6 shows that these decreases in the concentrations of proteins in plasma in response to Aroclor 1254 were accompanied by a significant increase ($P < 0.05$) in the plasma concentration of urea at 120 hr compared to controls, indicating increased protein breakdown.

DISCUSSION

Effects of Aroclor 1254 *on plasma and liver lipids*

Increased plasma concentrations of triacylglycerol and total cholesterol are well established responses to PCBs in humans [5–7] and in rats [8]. The small increases reported here were evident 120 hr after treatment (see Fig. 1), although greater changes would be expected over a longer period of time. The massive accumulation of triacylglycerol-like lipid droplets in the liver ('fatty liver syndrome') in response to Aroclor 1254 is also a well documented response of animals to many xenobiotics, including PCBs [7–9]. Although 'fatty liver' is regarded as a pathological condition, it was noticeable that pigeons that developed this condition in response to Aroclor 1254 had only developed mild hepatic necrosis (e.g. apoptosis), whereas pigeons with lower concentrations of hepatic lipids but without fatty liver had developed a more severe hepatic necrosis. This suggests that the development of a fatty liver could be protective for the liver and that sequestration of PCBs in the lipids that accumulate in the liver could diminish the effects caused by PCBs.

The mechanisms involved in these responses to PCBs are not well understood, but it is significant that pigeons that developed fatty liver did not develop a wasting syndrome and had a high hepatic glycogen content as judged by PAS staining. In addition, these pigeons had hepatic glutathione concentrations that did not differ from controls, but they were significantly higher than with pigeons that did

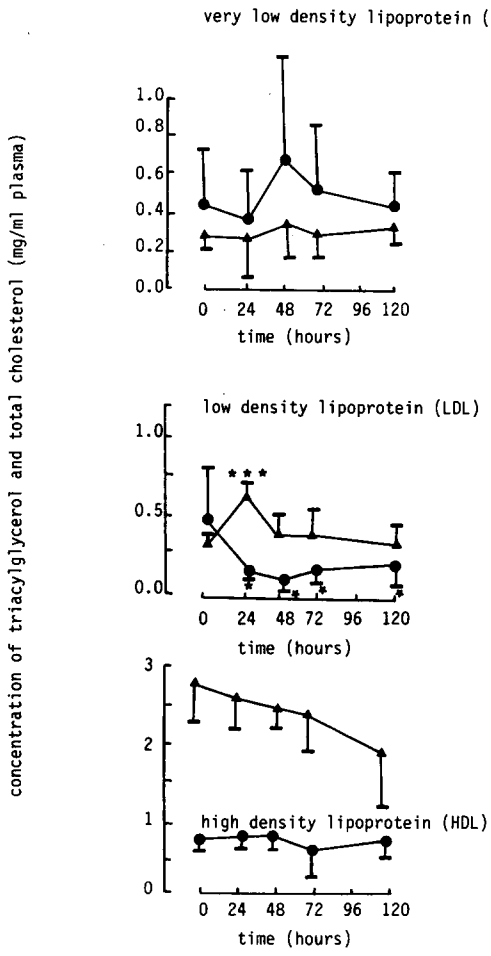

Fig. 2. Plasma concentrations of portomicron plus VLDL, LDL and HDL triacylglycerol and total cholesterol after injecting pigeons with Aroclor 1254. Pigeons were injected with 1.5 mmol of the commercial PCB mixture Aroclor 1254 and killed at the times shown. Details of the preparation of the plasma fractions and measurements of triacylglycerol (●) and total cholesterol (▲) are given in Materials and Methods. Values are means ± SD. The number of independent experiments are given in Fig. 1; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to mean values for controls at 24 hr (N = 4) and 120 hr (N = 5).

not develop a fatty liver in response to PCBs [4]. These results provide strong evidence for the protective effect of developing a fatty liver. The sequestration into hepatic lipid droplets of PCBs is likely to reduce their metabolism and, by implication, prevent a significant lowering of the hepatic concentration of glutathione due to enhanced metabolism of PCBs by cytochrome P450-dependent monooxygenases (high oxidative stress due to enhanced metabolism, for example, lowers hepatic concentrations of glutathione [4]).

Furthermore, studies by Dzogbefia *et al.* [10] have shown that feeding rats with a commercial mixture of PCBs (0.1% by weight of the feed) for 30 days increased the incorporation by liver homogenates of [^{14}C]*sn*-glycerol 3-phosphate into mono-, di- and triacylglycerols 2.4-, 14- and 10-fold, respectively, and increased the incorporation of [^{14}C]palmitate into triacylglycerol 70-fold. This increased synthesis of acylglycerols in liver in response to PCBs coincided with a substantial proliferation of the smooth endoplasmic reticulum [9, 11], as observed in the present experiments, which is the site of triacylglycerol synthesis. By contrast, Kling and Gamble [12] found that hepatic cholesterol synthesis from [^{14}C]acetate or from [^{14}C]mevalonate was depressed by about 28% in these circumstances. Quazi *et al.* [13] suggested that the rate of cholesterol synthesis in livers of rats fed the commercial PCB mixture Aroclor 1248 (0.03% by weight of the feed) for 7 days may be higher than the rate of cholesterol degradation to account for the higher concentration of cholesterol in the liver and plasma of treated rats compared with controls.

Kohli *et al.* [14] have shown that giving rats a single oral dose (0.13 mmol/kg body weight) of the toxic coplanar congener 3,3′,4,4′,5,5′,hexachlorobiphenyl (PCB 169) increased the concentrations of triacylglycerol and total cholesterol in liver 7- and 1.4-fold, respectively, after 72 hr. Under the same conditions, the less toxic congener 2,2′,3,3′,5,5′-hexachlorobiphenyl (PCB 133) did not lead to the accumulation of triacylglycerol in the liver and caused a small decrease in total hepatic cholesterol. This suggests that there may be specific molecular requirements (e.g. substitution with chlorine atoms at adjacent *meta–para* carbon atoms rather than in the *ortho*-position) for PCB congeners and isomers to elicit a toxic response and to produce 'fatty liver'. It will be of interest to know whether these individual PCBs increase the activity or the amount, or both, of hepatic enzymes involved in triacylglycerol, phospholipid, and cholesterol biosynthesis such as phosphatidate phosphatase (EC 3.1.3.4), diacylglycerol acyltransferase (EC 2.3.1.20) and hydroxymethylglutaryl-CoA reductase (EC 1.1.1.88).

Sandberg and Glaumann [15] have also shown that rats treated intraperitoneally with 0.15 mmol Aroclor 1254/kg body weight for 6–8 days had increased amounts of triacylglycerol, phospholipid, cholesterol and total lipid in their livers. The rate of incorporation of [^{14}C]glycerol into triacylglycerol and into total lipids by the hepatic microsomes from these rats was increased, but there was a decreased rate of incorporation into the triacylglycerol component of lipoporteins and into the total lipids associated with the Golgi apparatus. The authors suggested that Aroclor 1254 treatment had impaired the transport of VLDLs from the endoplasmic reticulum to the Golgi, which would result in decreased secretion of VLDLs from the hepatocytes. This would not conflict with the results described in Fig. 1, where increased plasma concentrations of triacylglycerol and of total cholesterol are reported, since we have evidence that PCBs inhibit the metabolism of secreted VLDLs by depressing significantly ($P < 0.05$) lipoprotein lipase activity (EC 3.1.1.34) (unpublished results).

Effects of Aroclor 1254 *on plasma proteins*

Decreased plasma concentrations of total protein, albumin, and total apolipoproteins (Fig. 4) 120 hr after treating pigeons with Aroclor 1254 suggests a

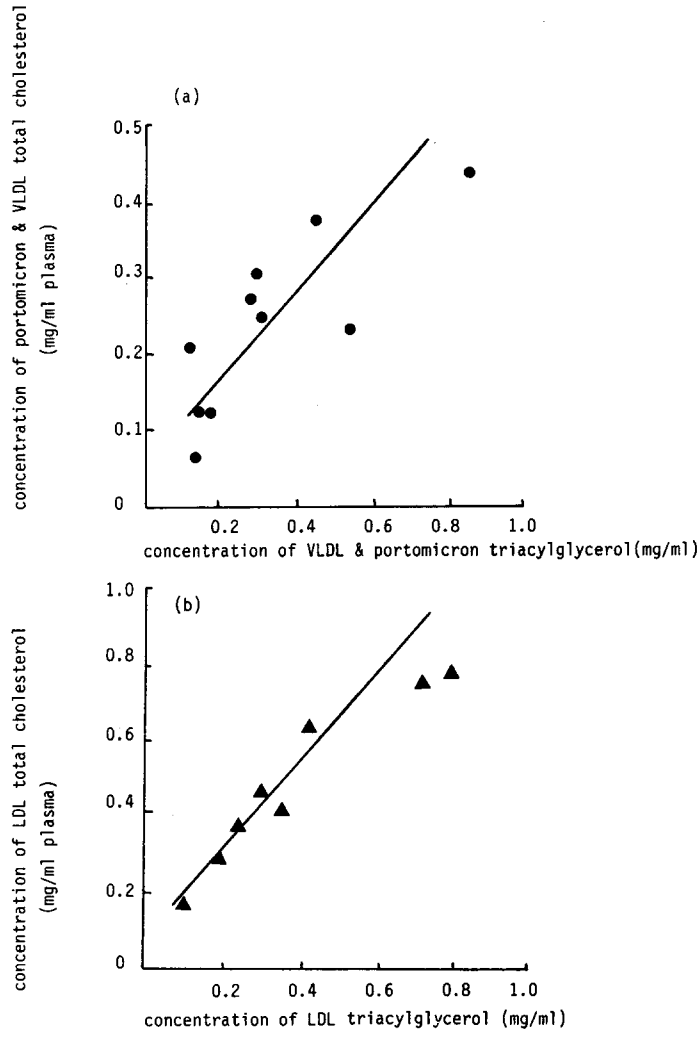

Fig. 3. Correlations between plasma concentrations of the triacylglycerol and total cholesterol components of (a) the portomicron plus VLDL fraction and (b) the LDL fraction 120 hr after injecting pigeons with Aroclor 1254. Pigeons were injected with 1.5 mmol of the commercial PCB mixture Aroclor 1254 and killed after 120 hr. Details of the preparation of the plasma fractions and measurement of triacylglycerol and total cholesterol are given in Materials and Methods. The values plotted are for individual pigeons. For (a) $r = 0.88$, $P < 0.001$; for (b) $r = 0.91$, $P < 0.001$.

decrease in hepatic protein synthesis, and an overall increase in protein breakdown is indicated by the increased plasma concentration of urea (Fig. 6). Decreased hepatic synthesis of apolipoproteins or their increased degradation, or both, would explain, at least in part, the accumulation of triacylglycerol-like material in the liver and the increased proportion per millilitre of plasma of triacylglycerol and total cholesterol compared to total apolipoproteins (cf. Figs 2 and 4) 120 hr after treatment. The proliferation of smooth but not rough endoplasmic reticulum in the liver in response to Aroclor 1254 also suggests increased lipid synthesis compared to protein synthesis.

There is an apparent paradox between the plasma concentration of portomicron plus VLDL apolipoproteins being significantly raised ($P < 0.05$) 24 hr after treatment of pigeons with Aroclor 1254 and then declining towards control values at 120 hr (see Fig. 5), and the impaired transport of VLDLs from hepatocytes in response to Aroclor 1254 suggested by Sandberg and Glaumann [15]. In the present study the increased plasma concentrations of VLDL are the result of a significant ($P < 0.05$) decrease with conversion of VLDLs to LDLs by lipoprotein lipase in response to Aroclor 1254 (unpublished results). This suppression is also reflected in the similar pattern of decrease in plasma concentrations of the triacylglycerol (Fig. 2b) and apolipoprotein (Fig. 5b) components of the LDL fraction in response to Aroclor 1254, indicating overall decreased LDL formation due to partial suppression of lipoprotein lipase activity.

The precise mechanism of an enhanced protein breakdown in response to PCBs is not understood. We have obtained preliminary evidence, however,

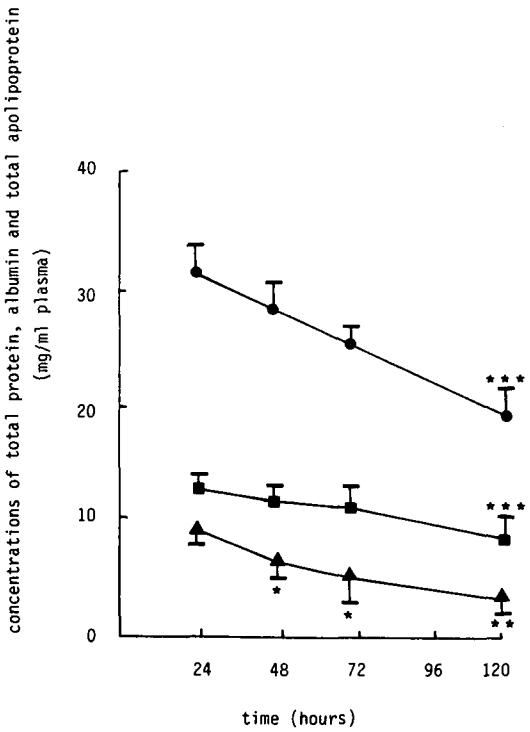

Fig. 4. Plasma concentrations of total protein, albumin and total apolipoproteins after injecting pigeons with Aroclor 1254. Pigeons were injected with 1.5 mmol of the commercial PCB mixture Aroclor 1254 and killed at the times shown. Details of the preparation of plasma and measurements of total protein (●), albumin (■) and total apolipoproteins (▲) are given in Materials and Methods. Values are means ± SD. The numbers of independent experiments are given in Fig. 1. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to mean values of controls at 24 and 120 hr.

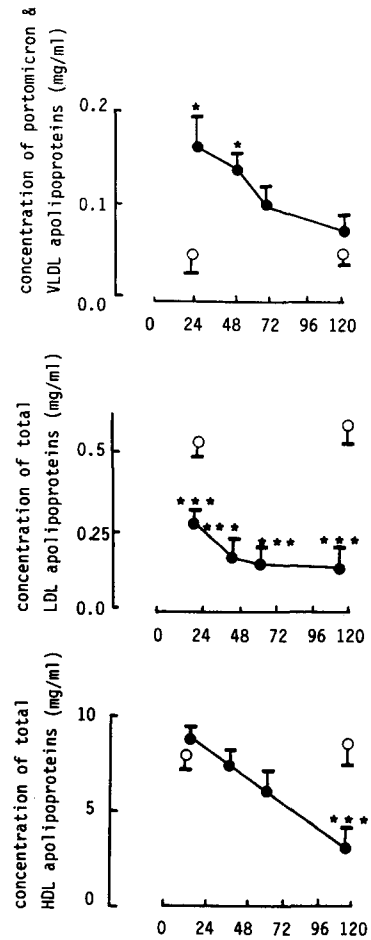

Fig. 5. Plasma concentrations of portomicron plus VLDL, LDL and HDL apolipoproteins after injecting pigeons with Aroclor 1254. Pigeons were injected with 1.5 mmol of the commercial PCB mixture Aroclor 1254 or with corn oil and killed at the times shown. Details of the measurement of the concentration of apolipoproteins in plasma are given in Materials and Methods. (●), Treated pigeons; (○), control pigeons. Values are means ± SD. The numbers of independent experiments are given in Fig. 1. * $P < 0.05$,. *** $P < 0.001$ compared to mean values of controls at 24 and 120 hr.

to suggest that the induction *in vivo* by PCBs of cytochrome P450-dependent monooxygenases is linked to an enhanced demand for essential amino acids and, in particular, for sulphur-containing amino acids. This demand for S-containing amino acids for the synthesis of induced cytochrome P450-dependent monooxygenases may necessitate the diversion of these amino acids from other biochemical processes in the cell and the enhanced breakdown of proteins to obtain essential amino acids [16]. It is of considerable interest that rats given an essentially S-amino acid-free diet develop a fatty liver and that raised serum cholesterol and triacylglycerol concentrations in PCB-treated rats can be restored to control values when these rats are given a diet rich in *S*-amino acids [17].

Proposed model for the transport of PCBs in plasma and their uptake by cells

A recent study by Soues *et al.* [18] has provided evidence for the binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benzopyrene with lipoproteins isolated intrahepatically. Thus, our identification of PCB isomer and congeners in lipoproteins isolated from serum, and by analogy, the identification of similarly highly lipophilic xenobiotics associated with intracellular lipoproteins has prompted us to propose the following model of cellular uptake of PCBs.

After ingestion of PCB-contaminated food, the individual PCB isomers and congeners absorbed are secreted into the bloodstream in association with hydrophobic components of chylomicra (or portomicron in the case of birds) and then in association with the VLDLs synthesized in the liver. These hydrophobic components include the triacylglycerol and cholesterol ester 'core', the phospholipid and cholesterol components of the lipid–plasma interface, and the hydrophobic domains of apolipoproteins. As chylomicra and VLDLs come in contact with the lipoprotein lipase located on the surface of the capillary endothelial cells of extrahepatic tissues, especially adipose tissues, PCBs are transferred into

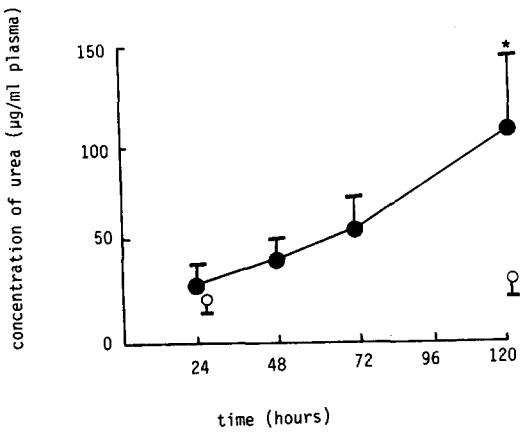

Fig. 6. Plasma concentration of urea after injecting pigeons with Aroclor 1254. Pigeons were injected with 1.5 mmol of the commercial PCB mixture Aroclor 1254 and killed at the times shown. Details of the preparation of plasma and measurement of urea are given in Materials and Methods. Concentration of urea in treated (●) and in controls (○). Values are means ± SD. The number of independent experiments are given in Fig. 1; * $P < 0.05$ compared to control values at 120 hr.

these cells [4]. Hormone-dependent mobilization of this material will be accompanied by the release of PCBs, though the rate of release of fatty acids may not be the same as that of PCBs [19], and conditions such as stress and starvation will increase PCB release from adipose tissue into the bloodstream [20]. The released PCBs associate mainly with the hydrophobic domains of plasma proteins, especially albumin, by non-covalent binding [21] but there may also be other PCB-binding proteins. Chylomicron 'remnants', as well as the IDLs derived from VLDLs, similarily release part of their stored PCBs to plasma proteins.

PCBs associated with LDLs derived via IDLs from VLDLs are taken up by cells, including hepatocytes, by receptor-mediated or receptor-independent endocytosis [22, 23] or by transport across the plasma membrane at rates that may be determined by the molecular structures of the individual PCBs [24]. Once inside the cell, the PCB–LDL complex is likely to be degraded by lysosomal enzymes with the release of PCBs. It will be of interest to know whether there are high-affinity hepatic intracellular PCB-binding proteins involved in transporting PCBs within the cell. There is recent evidence from immunoperoxidase staining studies for intracellular proteins in Clara cells of the lung that associate almost exclusively with PCBs [25]. Individual PCB isomers or congeners can then associate with the hydrophobic domains of a variety of intracellular molecules leading to the following effects:

(i) Intracellular transfer to the smooth endoplasmic reticulum results in the metabolism of certain PCB isomers and congeners, including electrophilic metabolites by the cytochrome P450-dependent enzymes of this membrane, as judged by metabolism *in vivo* of commercial mixtures of PCBs and of individual PCBs [4]. PCB isomers and congeners or their metabolites may bind to a receptor (i.e. the arylhydrocarbon (Ah) receptor) that controls gene expression and is implicated in the control of the different patterns of hepatotoxic and immunosuppressive effects produced by PCBs [26–29];

(ii) PCBs alter the lipid, protein and enzyme components of proliferating smooth endoplasmic reticulum [4, 27, 30, 31]. In addition, if PCBs bind at or near the active site of an enzyme, inhibition of enzyme activity might occur. By contrast if binding causes a conformational change in an enzyme, this might result in either activation or inhibition of the enzyme. Examples of the effects of PCBs include increased activities of cytochrome P450-dependent enzymes involved in drug metabolism, of fatty acid desaturases which produce an increased proportion of arachidonate in response to PCBs [4, 29, 30], and of enzymes [4, 30, 31] involved in triacylglycerol formation leading to 'fatty liver syndrome'. PCBs can decrease the synthesis or increase the degradation, or both, of apolipoproteins and albumin in the liver (see (iv) below);

(iii) PCBs transferred to organelles in hepatocytes involved in lipoprotein assembly and secretion could become incorporated into the lipid components and the hydrophobic domains of the apolipoproteins of lipoproteins as well as being complexed to the hydrophobic domains of albumin. The evidence reported by Soues *et al.* [18] has clearly shown that other xenobiotics are associated with lipoproteins intrahepatically. PCBs impair the transport of 'nascent' VLDLs from the rough endoplasmic reticulum to the Golgi apparatus and the exocytosis of these lipoproteins [15];

(iv) Decreasing the hepatic synthesis and secretion of VLDLs, HDLs and albumin leads to the observed decreased plasma concentrations of apolipoproteins and albumin. This coincides with increased protein breakdown, though it is not known which tissues are involved. We have recently obtained experimental evidence that increased plasma concentrations of the triacylglycerol and total cholesterol components of lipoproteins are linked to the suppression by PCBs of lipoprotein lipase activity (unpublished results).

(v) A number of individual PCBs are metabolized by hepatocytes to products that become toxic. To be detoxified, these need to be further metabolized in the liver. By contrast, the sequestration of PCBs in lipid droplets and their slow release via lipoprotein–PCB complexes could represent a less hazardous situation, since the metabolism by cytochrome P450 dependent monooxygenases is reduced, which in turn could prevent high oxidative stress to hepatocytes. Histopathological examination of the livers of PCB-treated pigeons has shown that the development of a fatty liver is linked to reduced cell necrotic events (e.g. mild apoptosis) and to a higher concentration of hepatic glycogen without signs of a wasting syndrome [4]. In addition, these animals had significantly higher concentrations of reduced hepatic glutathione compared with animals that did not develop a fatty liver in response to PCB. Thus there is strong evidence to suggest that the development of a fatty liver could be considered as an adaptation of an animal to avert some of the toxic effects produced by PCBs.

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