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Polychlorinated Biphenyl Uptake and Transport by Lymph and Plasma Components[1] (42073)

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Abstract. The uptake and vascular transport of ingested Aroclor 1242, an isomeric mixture of polychlorinated biphenyls (PCB), was investigated in experimental animals. High concentrations of ingested PCB were found in the chylomicron fraction of thoracic duct lymph. When the lymph flow was exteriorized PCB were not subsequently found in the vascular circulation. When lymph was not exteriorized plasma PCB concentrations reached maximal levels 6 hr after ingestion. Less than 1% of total plasma PCB was detected in cellular fractions of blood over a 10-hr period following ingestion. Chylomicrons contained 31% of total plasma PCB 30 min after ingestion, decreasing to less than 6% at 4 hr. A maximum of 10% of plasma PCB at 1 hr, and <5% at 6 hr, after ingestion was associated with very low density lipoproteins (VLDL) or low density lipoproteins (LDL). Although PCB enter the vascular circulation with the chylomicron fractions of lymph, delipoproteinated plasma contained 52% of the total PCB in blood collected 30 min after ingestion. This level increased to 78% after 2 hr, and remained constant at about 80% for an additional 8-hr period. High performance liquid chromatographic (HPLC) examinations of delipoproteinated plasma from blood taken 6 hr after PCB ingestion showed elution of >95% of plasma PCB to coincide with the albumin peak. Electrophoretic examinations of delipoproteinated plasma showed the association of PCB with albumin to be noncovalent. The results suggest that apolar PCB are absorbed into intestinal epithelial cells from which they are secreted into the lymphatic drainage sequestered within the apolar core of chylomicrons, that these PCB transit the thoracic duct and enter the vascular circulation within chylomicrons and are metabolized or otherwise released from chylomicrons during hepatic chylomicron clearance, and that resulting PCB or PCB derivatives circulate in association with plasma albumins.

Polychlorinated biphenyls (PCB) are persistent organochlorine compounds ubiquitously distributed as environmental pollutants. The chemical stability, relatively long biological half-life, and lipophilicity of these compounds result in their bioaccumulation in the food chain (1). Reviews of PCB toxicity describe chloracne as a major clinical indicator of human exposure (2–9). Clinical presentations in humans and animals include anorexia, loss of weight, severe diarrhea, decreased reproductive efficiency with fetal wastage, immune dysfunction, and nervous system dysfunction with terminal ataxia. Various of the PCB isomers are inducers of microsomal mixed function oxidase (MFO) enzymes which metabolize PCB through reactive oxide forms to polar products which can be excreted (10, 11). Their known toxicity, bioaccumulation in the food chain, and persistence as environmental pollutants have combined to stimulate extensive research into the distribution, metabolism, excretion, and storage of PCB.

It has been widely assumed that ingested PCB are absorbed into the bloodstream, transported to the liver, and metabolized by hepatic MFO enzyme systems, and that eventual disposition of PCB in an animal is dependent on the polarity of the compounds (12, 13). The polarity of PCB, which dictates their metabolism, stability in tissue reservoirs,

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and ultimate metabolism and excretion, is dependent on the extent of their chlorination. Tetra-, penta-, and hexachlorinated biphenyls are more nonpolar, are stable in tissues for a longer period of time, and are metabolized and excreted less readily than are di- and trichloro forms (13). Extensive investigation has been directed toward understanding PCB metabolism, storage, excretion, and toxicity, and has resulted in the observation that lipoproteins play a major role in vascular transport of the highly lipophilic hexachlorobiphenyl isomers (14, 15). However, data are not available on the mechanisms of uptake and transport of the less highly chlorinated, more polar, di- and trichloro forms of PCB.

Lipoproteins are reported to partition lipophilic compounds, such as polyhalogenated hydrocarbon insecticides (16–19), polychlorinated biphenyls (19), and benzo[*a*]pyrene (20–22), *in vitro.* Lipoproteins have been proposed to be the agents of transport of lipophilic compounds in the blood vascular system (23–25); however, *in vivo* studies delineating the uptake and vascular transport of PCB are limited (15, 26). Although it would be logical to assume that PCB, being lipophilic, enter the gastrointestinal lymphatic drainage with dietary fats and are transported intravascularly in association with lipoproteins, unified data verifying this assumption are not available.

In this paper we present a study of the *in vivo* uptake and vascular transport of ingested Aroclor 1242. We show that ingested PCB are initially associated with circulating chylomicrons, and that chylomicron-associated PCB are rapidly cleared from the vascular circulation concomitant with the appearance of presumed PCB metabolites noncovalently associated with plasma albumin.

Materials and Methods. Four 1-year old male Black Labrador dogs weighing 23 to 25 kg were used for this study. The animals were fasted overnight, and each animal was fed 25 μCi of an isomeric mixture of polychlorinated biphenyls (Aroclor 1242, ^{14}C-PCBs, 24.4 mCi/mmole, New England Nuclear; radiochemical purity of ^{14}C-PCBs was determined, by column chromatography on alumina, to be greater than 99%) mixed with 370 ml of evaporated milk. Blood was drawn from an indwelling silastic catheter in the jugular vein into a heparinized syringe at 30-min intervals for a maximum of up to 10 hr after ingestion of the PCBs. Water was provided *ad libitum.* Concentrations of ^{14}C-PCBs in plasma fractions were determined radiometrically.

Separation of lipoproteins by centrifugation. Lipoproteins, including chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL), were isolated from plasma using the KBr density centrifugation procedures of Rudel *et al.* (27), and the preparative ultracentrifugation procedures of Hatch and Lees (28). Residual proteins in delipoproteinated plasma included the mixture typically known as albumin. Concentrations of ^{14}C-PCBs in plasma lipoprotein classes and in residual proteins were determined radiometrically. Recovery was expressed as the percentage of ^{14}C-PCBs in each plasma component class compared to the total ^{14}C-PCBs in an aliquot of plasma.

Separation of lipoproteins using high performance liquid chromatography. A high performance liquid chromatographic (HPLC) separation of lipoproteins was completed on intact and on delipoproteinated plasma using the procedures of Busbee *et al.* (29), which employed TSK 4000 SW modified silica gel size exclusion columns. Plasma was eluted through the HPLC system at 1 ml/min using sodium phosphate buffer (0.02 *M,* pH 6.8) containing 0.02% NaN_3. The column effluent was monitored by spectral analysis at 254 nm, and 0.25-ml elution fractions were collected. The ^{14}C-PCB concentration in each fraction was determined radiometrically.

SDS–polyacrylamide gel electrophoresis. Plasma samples from blood taken after ingestion of PCBs were examined using the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) procedures of Weber *et al.* (31). The gels were stained, and relative electrophoretic mobilities (R_f) of plasma proteins were calculated. The gels were sliced into 2-mm fractions, and the ^{14}C-PCB content of each fraction was determined radiometrically.

Thoracic duct cannulation. Cannulation of the thoracic duct was completed using the thoracic duct fistula method (30). The left jugular vein was dissected down on its medial border and small branches and entering veins

were tied down to the level of the innominate vein. The external jugular was ligated near the upper border of the thyroid cartilage and the vein was sectioned below. Sterile, size 20, polyvinyl tubing was tied into the vein and exteriorized through the skin. The animal tolerated the procedure well, and was eating and drinking normally within hours. After 2 days of recovery the animal was treated with ^{14}C-PCB in evaporated milk as described above. The concentration of PCBs was radiometrically determined for lymph collected from the thoracic fistula cannula and for blood collected by venipuncture as given above.

Results. Concentrations of PCB in lymph, plasma, and cellular fractions of blood were determined at specified intervals following ingestion of ^{14}C-PCBs by experimental animals. PCB concentrations in lymph were elevated as early as 30 min after ingestion, peaking at 1 hr, and returning to near baseline concentrations 4 hr after ingestion (Fig. 1). When the lymph flow was not returned to the vascular circulation, blood concentrations of PCB remained at baseline levels (Fig. 1). Fasted animals did not show chylomicrons in either lymph or plasma until they were fed a high-fat meal, after which chylomicrons appeared in the lymph and subsequently in the blood plasma (data not given). A determination of PCB concentrations in chromatographically separated components of lymph from an animal fed a high fat meal showed that PCB eluted from the column coincident with the lymph chylomicron fraction (Fig. 2). When lymph flow was returned to the vascular circulation, plasma PCB concentrations peaked 6 hr after ingestion (Fig. 3). Less than 1% of PCB in blood samples was detected in the cellular fractions over a 10-hr postingestion period. PCB concentrations in chylomicrons, VLDL, LDL, and delipoproteinated plasma were determined and are shown in Fig. 4. Chylomicrons contained 31% of total plasma PCB 30 min after ingestion by the test animals, decreasing to 18% at 1 hr, 9% at 2 hr, and less than 6% at 4 hr. Delipoproteinated plasma from blood collected 30 min after ingestion contained 52% of total plasma PCB. This value increased to 78% after 2 hr, and remained constant at around 80% for an additional 8-hr period. Less than 7% of the total plasma PCB concentration was associated with VLDL or LDL over the 10-hr period.

An HPLC examination of plasma fractions

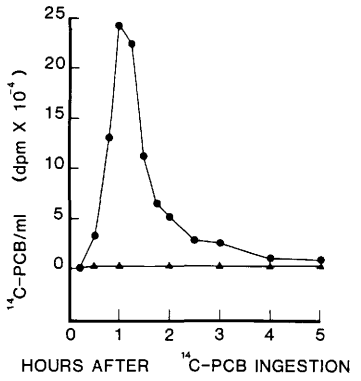

FIG. 1. An analysis of ^{14}C-PCB uptake into lymph. Experimental animals were surgically prepared to drain lymph from a thoracic duct fistula. After recovery, animals were fasted 24 hr, a cannula was inserted into the thoracic duct fistula, and the animals were fed ^{14}C-PCB (Aroclor 1242) in evaporated milk. Lymph and blood were collected at indicated intervals after PCB ingestion. PCB concentrations in lymph and plasma were radiometrically determined. In animals where the normal lymph flow was exterioized, PCB were found in lymph but not in plasma. ^{14}C-PCB in lymph (●); ^{14}C-PCB in plasma (▲).

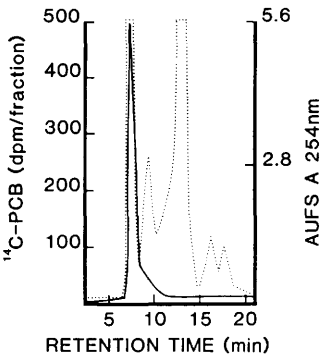

FIG. 2. An examination of ^{14}C-PCB distribution between lymph components. Experimental animals were fasted 24 hr and fed PCB in evaporated milk. Lymph was collected from a cannulated thoracic duct fistula at 1 hr and 0.5 ml of lymph was applied to a TSK4000 SW HPLC column for size exclusion separation. Fractions of lymph (0.25 ml) were collected at elution and were radiometrically assessed for PCB. Chylomicrons elute at the column void volume, 7.0 min, while LDL elutes at about 9.3 min, and albumins elute at 12–13.5 min. A 254-nm profile (---); ^{14}C-PCB dpm/elution fraction (—).

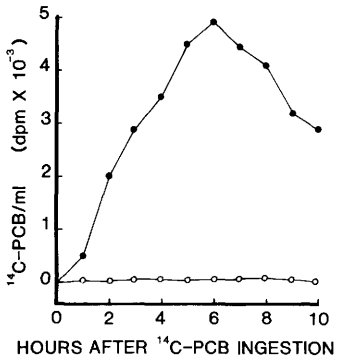

FIG. 3. Plasma levels of circulating ^{14}C-PCB over a 10-hr period after PCB ingestion. Experimental animals were fed PCB in evaporated milk and the lymphatic flow was not exteriorized. Blood taken after ingestion of ^{14}C-PCB by the test animals was separated into plasma (●) and cellular (○) fractions, and the presence of PCBs was radiometrically determined.

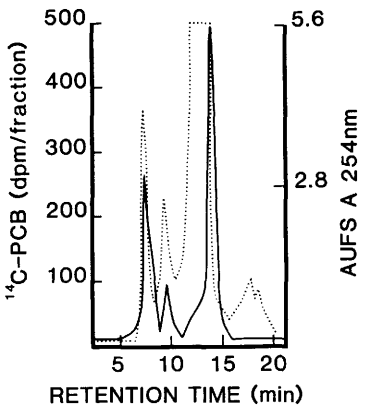

FIG. 5. The distribution of ^{14}C-PCB between plasma components 1 hr after ingestion of PCB. Fasted experimental animals were fed PCB and the lymphatic flow was not exteriorized. A blood sample taken 1 hr after ingestion was centrifuged to remove cells and 0.5 ml of plasma was applied to a TSK4000 SW HPLC size exclusion column as given under Materials and Methods. Elution fractions were radiometrically assessed for PCB distribution between plasma components. Chylomicrons elute at the void volume, 7.0 min, LDL elutes at about 9.3 min, and albumin elutes from 12–13.5 min, overlapping with HDL at about 10.5–12 min. A 254-nm profile (---); ^{14}C-PCB dpm/elution fraction (—).

from blood taken 1 hr or 6 hr after PCB ingestion showed significant differences in PCB distribution into plasma components. The 1-hr sample shows elevated levels of chylomicrons eluting at the void volume. PCB were distributed between the chylomicron and albumin peaks, with a small PCB peak eluting with LDL (Fig. 5). At 6 hr (Fig. 6) chylomicrons had been cleared from the vascular circulation and no PCB were found in the column void volume fractions (7.0–7.2 min), or in LDL fractions. The major peak of PCB (>95% of total PCB in the plasma sample) eluted at 13.25 min, which corresponds to the albumin peak. HDL and albumin elute together in overlapping bands with retention times between 10 and 13.5

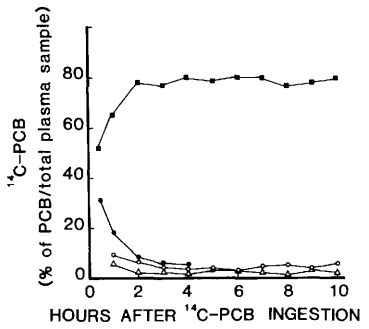

FIG. 4. Percentage of total circulating ^{14}C-PCB in plasma components. Experimental animals were fed PCB in evaporated milk and the lymphatic flow was not exteriorized. Plasma from blood taken after ingestion of PCB was centrifugally separated into chylomicron (●), VLDL (○), LDL (△), and delipoproteinated (■) fractions. PCB were radiometrically determined for each fraction and expressed as the percentage of total plasma PCB levels.

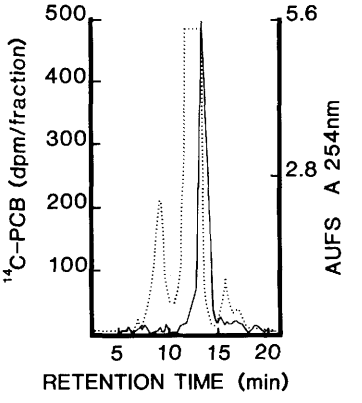

FIG. 6. The distribution of ^{14}C-PCB between plasma components 6 hr after ingestion of PCB. The experiment was completed as shown in Fig. 5, but blood samples were taken at 6 hr. Elution of plasma components was spectrally monitored and fractions eluted from the column were assessed for the presence of PCB. A 254-nm profile (---); ^{14}C-PCB dpm/elution fraction (—).

min, respectively. To confirm that HDL and PCB elution times did not coincide, the HPLC system was used to examine delipoproteinated plasma from blood taken 6 hr after ingestion (Fig. 7). Delipoproteinated plasma exhibited residual proteins, with retention times between 11 and 13.5 min, eluting in a major peak containing the mixture of plasma components typically referred to as albumin. The retention time of labeled PCB, (recovery >95%), carried by delipoproteinated plasma coincided with the albumin elution peak. These data suggest that parent PCB enter the intestinal lymphatic drainage sequestered in chylomicrons, and that the chylomicrons and associated parent PCB are rapidly cleared from the blood concomitant with an increase in PCB circulating with plasma albumins.

To determine if PCB were covalently bound to plasma albumin, we assessed their comigration with proteins from plasma collected 6 hr after their ingestion by test animals. Relative electrophoretic mobilities of plasma proteins were compared to labeled PCB concentrations in 2-mm gel slices (Fig. 8). No PCB were detected in the albumin band ($R_f = 0.46$). Rather, they were recovered at the end of the resolving gel ($R_f = 1.00$), suggesting that PCB circulating with plasma were not covalently bound to albumin.

Discussion. Aroclor 1242 is an isomeric mixture of polychlorinated biphenyls which average 42% chlorine by molecular weight and have an average of three chlorine residues per molecule. We anticipated that this PCB would be lipophilic and that, following ingestion, it would be absorbed into the intestinal lymphatic drainage as was reported by Ziprin *et al.* (26) for PCB in sheep. The presence of ingested PCB in lymph chylomicrons after administration suggests that ingested PCB may be synthesized into, or sequestered within, chylomicrons within intestinal epithelial cells, secreted into the intestinal lymphatic drainage via the lacteals, and ultimately found in the thoracic duct lymphatic flow. Exteriorization of the thoracic duct lymph flow prevents entry of both chylomicrons and PCB into the vascular circulation. Absence of PCB in the vascular circulation under these conditions indicates that PCB with lipophilicity equal to or greater than Aroclor 1242 are not absorbed into the intestinal blood vascular drainage.

Examinations of PCB uptake and distribution when lymph flow was not exteriorized showed that Aroclor 1242, in the unmetabolized form administered to test animals, was not distributed to any great extent into VLDL, LDL, HDL, or cellular fractions of blood. Rather, PCB in the vascular circulation were initially associated with chylomicrons after ingestion. Circulating plasma chylomicrons are typically partially hydrolyzed by extrahepatic lipases found in the vascular endothelium and resulting chylomicron remnants are cleared from circulation by hepatic lipases (32, 33). Under the conditions of this

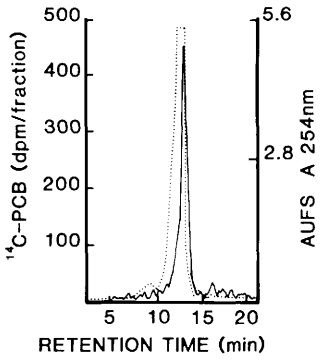

FIG. 7. The association of ^{14}C-PCB with components of delipoproteinated plasma from blood taken 6 hr after PCB ingestion. Delipoproteinated plasma from the test animals was examined by size exclusion HPLC analysis. Elution of plasma components was spectrally monitored and fractions eluted from the column were assessed for the presence of PCB. A 254-nm profile (—); ^{14}C-PCB dpm/elution fraction (---).

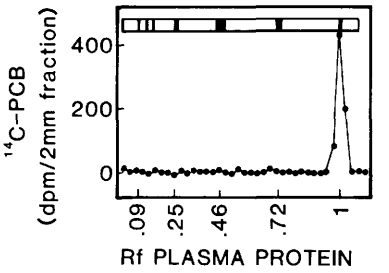

FIG. 8. An SDS–PAGE examination of the association of ^{14}C-PCBs with plasma proteins. Plasma from blood taken 6 hr after PCB ingestion by the test animals was separated using SDS–PAGE. The gels were cut into 2-mm slices and concentrations of ^{14}C-PCBs were radiometrically assessed.

study, an increase in the concentration of polar PCB circulating in association with albumin occurred concomitantly with a decrease in chylomicron-associated PCB in the vascular circulation. This suggests that PCB carried by chylomicrons were metabolized or otherwise released during hepatic clearance of chylomicrons and that resulting PCB subsequently circulated in the vascular system bound to plasma albumin. The disappearance of PCB associated with circulating chylomicrons and appearance of free PCB in the vascular circulation, presumed to be metabolites, are consistent with published data showing that dogs metabolize PCB rapidly, clearing them from circulation (34). We observed that parent PCB used for this study were adsorbed to the TSK4000 SW size exclusion column packing, and were not eluted from the column using polar solvents. Neither the PCB found in lymph or plasma chylomicron fractions, nor the labeled compounds, presumed to be PCB metabolites, circulating in association with plasma albumins from blood taken 6 hr after ingestion of the compounds were retained on the TSK4000 column. Separate explanations may exist for these phenomena. Partitioning studies indicate that lipophilic compounds, such as PCB, enter the apolar core of lipoproteins, such as chylomicrons, and would thus not be available for adsorption to the hydrophobic column matrix. Secondly, labeled compounds bound to albumins may be metabolites whose increased polarity and affinity for albumins resulted in their elution from the HPLC column with polar solvents. PCB-derived labeled compounds circulating with plasma albumin did not comigrate with albumin on SDS–PAGE, suggesting that they were not covalently bound. It is unlikely that compounds with the molecular weight of PCB would elute from the size exclusion column with a retention time characteristic of albumins in the absence of a physical attraction, such as electrostatic interactions, between the compounds and albumin. This work does not address the question of PCB metabolites or of the mechanism of PCB binding to albumins.

Maliwal and Guthrie (19) reported the *in vitro* partitioning of 2,4,5,2′,4′,5′-hexachlorobiphenyl (6-CB) into plasma LDL, while Vomachka *et al.* (14) showed the *in vitro* distribution of 6-CB among plasma VLDL, LDL, HDL, and residual proteins. Vomachka *et al.* (15) also reported the *in vivo* uptake of 6-CB into LP, with greatest initial uptake by LDL, and the transfer of 6-CB from LP to non-LP components with time. The data for 6-CB uptake and transport were expected to differ from data obtained in this study of Aroclor 1242 uptake and transport *in vivo*. Polarity of polychlorinated biphenyls, hence their lipophilicity and mode of uptake and transport, is dependent on the extent of chlorination of the biphenyl molecule (13). Hexachlorobiphenyl is expected to be more nonpolar than a PCB mixture with an average of three chlorine residues such as that used in this study. Although Aroclor 1242 is less lipophilic than is 6-CB, we expected that parent, nonmetabolized, PCB used in this study would be lipophilic, and would distribute from chylomicrons to other plasma lipoproteins after entering the vascular system, as was reported by Vomachka for 6-CB (15). This was not observed. Rather, this *in vivo* examination showed about a 7% transient uptake of parent Aroclor 1242 into any of the classes of lipoproteins other than chylomicrons.

The findings of this study suggest that ingested PCB, in this instance Aroclor 1242, are absorbed into the intestinal lymphatic drainage sequestered in chylomicrons. The data further suggest that PCB transported by chylomicrons enter the vascular circulation, are metabolized or otherwise released at the time of hepatic chylomicron clearance, and that the resulting free derivatives of PCB circulate associated with plasma albumin.

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