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INTRACELLULAR LIPOPROTEINS AS CARRIERS FOR 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN AND BENZO(*a*)PYRENE IN RAT AND MOUSE LIVER

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Abstract—The possible role of hepatic lipoproteins as intracellular carriers in the transport of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benzo(*a*)pyrene was assessed by *in vitro* and *in vivo* studies. Following administration of [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin or unlabelled 2,3,7,8-tetrachlorodibenzofuran to C57 BL/6 mice or Sprague–Dawley rats these compounds were bound to lipoproteins which subsequently underwent rapid and pronounced degradative processing, possibly catalysed by lipoprotein lipase, to heavier entities. At the highest doses of xenobiotics administered, an almost complete disappearance of lipoprotein particles was observed. The *in vitro* incubation of [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin-lipoprotein and [^{3}H]benzo(*a*)pyrene–lipoprotein complexes with separated *Ah* receptor and 4S protein, respectively, demonstrated that a passive transfer occurred; the latter was likely dependent on both the relative affinities of the ligands towards the different cellular binding components as well as on their quantitative binding capacity. Taken together, these findings support the idea of a carrier-role for lipoproteins in the intracellular transport of hydrophobic xenobiotics and it may be asked whether the widespread modulators of lipoprotein level such as fibrates or others affect drug transfer or action.

Many lipophilic xenobiotic compounds are carried in the bloodstream by plasma lipoproteins; such is the case for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)† [1, 2], benzo(*a*)pyrene (BP) [3–5], 3-methylcholanthrene [6], *p*-dimethylaminoazobenzene [7], etc. It has been demonstrated that BP is preferentially associated, in human plasma, with very low-density lipoproteins (VLDL) (29%) and low-density lipoproteins (LDL) (57%) and, to a lesser extent, with high-density lipoproteins (HDL) (10%) [3, 7]. In contrast, serum albumin plays a minor role in the transport of unmetabolized benzo(*a*)pyrene; 5% of the latter associates with albumin while 95% associates with lipoproteins [5]. The incorporation of BP [4] and TCDD [2] by cultured cells has been assessed using either normal human embryonic lung or skin fibroblasts, or with fibroblasts from patients with homozygous familial hypercholesterolemia, these are either LDL-receptor positive or LDL-receptor negative, respectively. The data showed that, if a significantly greater TCDD uptake from LDL by the normal cells occurred [2], the BP entered the cells from LDL despite the absence of specific receptor, by a rapid redistribution between the lipoprotein and cell membrane. As shown in the companion paper [8] the hepatic cytosol from various animal species contains a large spectrum of lipoproteins including VLDL, LDL, HDL as well as heavier lipoprotein entities.

Knowing that plasma lipoproteins have an important carrier-role for lipophilic xenobiotics in the blood, studies have been conducted to determine whether intracellular lipoproteins are able to play a similar function within the cells.

In this report, we present data showing that (i) subsequent to the *in vivo* binding of TCDD or 2,3,7,8-tetrachlorodibenzofuran to intracellular lipoproteins the latter undergo many changes and a catabolic processing leading, for the higher doses of xenobiotic, to an almost complete disappearance of lipoprotein particles and (ii) that transfer, by passive redistribution, of either [^{3}H]TCDD or [^{3}H]BP occurs *in vitro* to the *Ah* receptor or the 4S protein, respectively.

MATERIALS AND METHODS

Chemicals. Unlabelled 2,3,7,8-tetrachlorodibenzofuran and TCDD, as well as [^{3}H]TCDD (52 Ci/mmol), were purchased from KOR isotopes (Cambridge, MA); the radiochemical purity of the tritiated compound, > 98%, was checked after storage and, if necessary, the removal of radiolysed [^{3}H]TCDD was carried out by high performance liquid chromatography according to the procedure of Gasiewicz and Neal [9]. [G-^{3}H]benzo(*a*)pyrene (50.5 Ci/mmol), radiochemical purity 96.1% was purchased from Amersham (Bucks, U.K.). Hepes, dextran (M_r 150,000), activated charcoal, sodium phenobarbital, lipoprotein lipase were obtained from Sigma Chemical Co. (Poole, U.K.). Dithiothreitol and dimethyl sulfoxide were purchased from Merck

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† Abbreviations used: BP, benzo(*a*)pyrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; VLDL, very low-density lipoproteins; LDL, low-density lipoprotein; HDL, high-density lipoproteins; TCPOBOP, 1,4-bis [2-(3,5-dichloropyridyloxy)]benzene; TCDBF, 2,3,7,8-tetrachlorodibenzofuran; Hepes, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; LDC, light density component; B6, C57Bl/6N Cr/BR mice; LPL, lipoprotein lipase.

A.G. (Darmstadt, F.R.G.). Sudan black B was obtained from Aldrich Chemical Co. (Gillingham, U.K.). 1,4-Bis [2(3,5-dichloropyridyloxy)]benzene was a gift of Dr A. Poland (Madison, WI).

Animals. The animals were purchased from the following sources: C57 BL/6NCr/BR mice from Charles River (Cléon, France); Sprague–Dawley rats from Janvier (Le Genest, France). The mice (20 g) were pretreated by TCPOBOP and received, by intraperitoneal route, 3 mg/kg of compound dissolved in sunflower oil and they were killed 72 hr after treatment. The rats were pretreated, by phenobarbital (80 mg/kg), dissolved in 0.9% NaCl, and were injected intraperitoneally for three consecutive days before the animals were killed on day 4 for removal of the liver. As described previously, treatment of animals with either phenobarbital (rat) or TCPOBOP (mice) increases the hepatic levels of binding components including lipoproteins and improves their study [10, 11].

In vivo [^{3}H]TCDD *and unlabeled TCDBF treatment of rats and mice.* Two male rats (200 g) and three male B6 mice were injected intraperitoneally with 18 and 3.6 μg (0.282 and 0.570 μmol/kg body wt) [^{3}H]TCDD, respectively. The vehicle was 20 μl of *p*-dioxane for treatment of mice and a mixture of 10 μl *p*-dioxane in 500 μl of sunflower oil for treatment of rats. Untreated animals received the vehicle alone. Animals were killed 2 hr after injection, and the livers were perfused and homogenized as described for cytosol preparation (see below). Unlabelled TCDBF 10–1000 μg/kg body wt) was administered intraperitoneally to different groups of three B6 mice. The compound was dissolved in *p*-dioxane and 10 μl/10 g body weight was injected into mice. Untreated mice received the vehicle alone. Mice were killed 20 min to 30 hr after injection, and the livers were perfused and homogenized as described for cytosol preparation (see below).

Buffers and solutions. The standard buffer used for the preparation of cytosols and the binding experiments was HEDG: 25 mM Hepes, 1.5 mM EDTA, 1 mM dithiothreitol and 10% glycerol (v/v), pH 7.6. Potassium bromide solutions for analysis of lipoproteins by density gradient ultracentrifugation were prepared with solid KBr previously dried by heating at 120° for 16 hr; then the salt was dissolved in 0.9% NaCl, 0.01% EDTA solution pH 7.4. Sudan black solution, containing 0.1 g of dye dissolved in 100 ml ethylene glycol, was stored in a brown bottle at room temperature.

Preparation of cytosol. Mice were killed by cervical dislocation, and rats were killed by ether anesthesia. The liver was perfused, *in situ*, with cold 0.9% NaCl solution via a needle inserted into the heart, and then via the inferior vena cava with HEDG buffer. After extensive perfusion, the liver was removed, rinsed with 0.9% NaCl solution and HEDG buffer, minced, and homogenized in HEDG buffer (3 ml/g of liver) with a Teflon–glass homogenizer. All procedures were performed at 4°. The homogenate was centrifuged at 9,000 *g* for 20 min, and the resulting supernatant was centrifuged at 105,000 *g* for 1 hr. Cytosol was carefully drawn off without disturbing the surface lipid layer or the microsomal pellet. Protein concentrations were determined by the method of Lowry *et al.* [12] with bovine serum albumin as the standard; blank controls were prepared in HEDG buffer for each determination. Cytosol samples, pooled from several animals (2 to 20), usually contained 20–25 mg of protein/ml. They were used after preparation or stored either in liquid nitrogen or at −70°, for periods of up to 6 months before use in lipoprotein separation or binding experiments with *Ah* receptor and 4S protein; no loss of activity occurred under these conditions.

Preparation of light lipoproteins, Ah *receptor and 4S protein.* Aliquots (300 μl) of undiluted cytosol samples from B6 mice or rats were layered onto eight linear sucrose density gradients (5–20%) prepared in HEDG buffer. One of the eight samples was previously incubated with 10 nM [^{3}H]TCDD (for binding to the *Ah* receptor) and 10 nM [^{3}H]benzo(*a*)pyrene (for binding to the 4S protein) for 1 hr at 0–4°; the radioligands were added to cytosol (1 ml) in 10 μl dimethyl sulfoxide. After incubation, unbound and loosely bound radioligands were removed by adding cytosol sample to a dextran–charcoal pellet (10 mg of charcoal/mg of dextran, pelleted from HEDG buffer); dextran–charcoal was resuspended in the cytosol on a vortex mixer, incubated with the sample for 15 min, and then removed by centrifugation at 4000 *g* for 15 min. Gradients (4.8 ml) were centrifuged at 4° for 2 hr 40 min in a vertical tube rotor (Beckman VTi-65, g_{av} = 372,000) at 60,000 rpm. After centrifugation twenty-two 232 μl fractions (eight drops/fraction) were collected from the labeled sample with a Beckman recovery system. Radioactivity in each fraction was determined by liquid scintillation counting (Aquasol 2 from New England Nuclear, Boston, MA). According to the radioactivity profile, which indicates the position of the various components in the sucrose gradient, the fractions containing the non-labeled cytosolic components were pooled: as previously demonstrated [10], fractions 1 to 4 contain a mixture of light lipoproteins (VLDL and LDL), named LDC (light density component), fractions 6 to 12 contain the 4S protein and fractions 15 to 22 contain the *Ah* receptor. Concentration of various separated components was carried out by centrifugation at 3,000 rpm for 30 min (for LDC), 60 min (for 4S protein) or 90 min (for *Ah* receptor) in centriflo membrane cones CF25 (Amicon). Concentrates were removed with a pasteur pipette; then recovery of material adhering to the cone was maximized by rinsing with a small amount of HEDG buffer. Controls for cross-contamination for each separated component were made as previously described [10], and were satisfactory (not illustrated). The samples were kept at −70° and no loss of binding capacity occurred under these conditions for periods of up to 6 months.

Separation and analysis of lipoproteins. Potassium bromide (KBr) density gradient ultracentrifugation in a vertical rotor was used: cytosol samples were incubated with [^{3}H]TCDD and/or Sudan black solution in conditions described in the figure legend, then treated with dextran–charcoal for labeled samples. The density of samples (1.75 ml) was adjusted to 1.30 g/ml by the addition of dried solid KBr (550 mg); then 1.5 ml aliquots were loaded in the bottom of 5 ml Quick-seal tubes, and a discontinuous

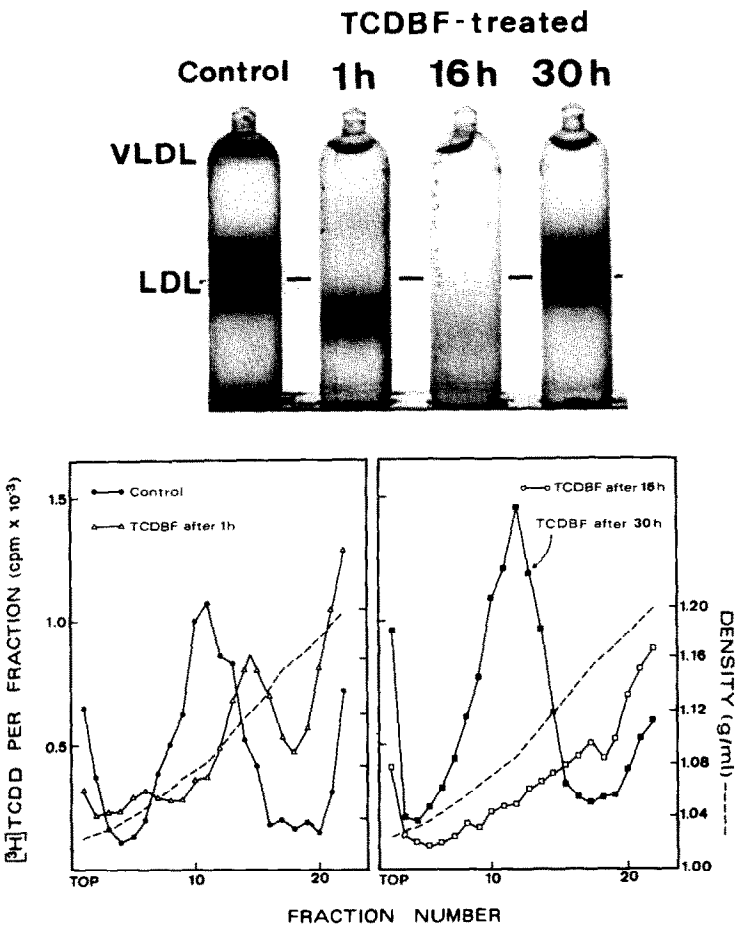

Fig. 1. Processing of lipoproteins from liver cytosol after treatment of mice with 2,3,7,8-tetrachlorodibenzofuran. TCDBF (1000 μg/kg body wt) was administered, intraperitoneally, to three groups of three B6 mice. The compound was dissolved in *p*-dioxane and 10 μl/10 g body weight was injected to mice. Mice were killed 1 hr, 16 hr or 30 hr after injection and the livers were perfused and homogenized for cytosol preparation as described in Materials and Methods. A group of three mice (control group) received the vehicle alone; the animals were killed 16 hr after injection and the liver cytosol prepared. 0.5 ml samples of cytosols were incubated with 10 nM [^{3}H]TCDD for 30 min at 4°. Following dextran-coated charcoal adsorption, 300 μl aliquots were prestained with 250 μl Sudan black solution for 30 min at 4° in the dark. The samples were diluted to 1.75 ml with HEDG then the density was adjusted to 1.30 g/ml by the addition of solid KBr (550 mg). 1.5 ml aliquots were examined by KBr density gradient ultracentrifugation in vertical rotor as described in Materials and Methods.

KBr density gradient was formed by overlayering 3.5 ml of 0.9% NaCl solution. Tubes were placed in a vertical rotor (Beckman VTi 65) immediately after the preparation of the gradient and centrifuged at 4° for 105 min at 63,000 rpm. After centrifugation, 22 fractions were collected from each gradient. Radioactivity in each fraction was determined by liquid scintillation counting and the position of stained lipoprotein bands was monitored spectrophotometrically at 610 nm.

Binding experiments. Cytosol samples (1 ml) were incubated with [^{3}H]TCDD for 1 hr at 0–4°. The radioligand was added in 10 μl dimethyl sulfoxide; dimethyl sulfoxide also was used as the solvent for nonradioactive TCDD in competition experiments. In the latter, an amount of solvent equal to that used to introduce the competitor was added to the control (no competitor) sample. Removal of unbound and loosely bound radioligands as well as sucrose gradient analysis of samples (300 μl) were carried out as described above.

Radioligand transfer experiments. The transfer of radioligands from lipoproteins to binding proteins was carried out by incubating two ml of cytosolic lipoprotein samples (LDC) with either 30 nM [^{3}H]TCDD or 100 nM [^{3}H]benzo(*a*)pyrene, respectively, for 30 min at 0–4°. It has been previously demonstrated that the rate of association for radioligands with lipoproteins was very rapid, since 80% of total binding occurred at 0° within 1 min [10]. The radioligands were added to the samples in 20 μl dimethyl sulfoxide. Unbound and loosely bound radioligands were removed by dextran–charcoal as described above, then 0.4 ml aliquots were kept in the ice for radioligand binding control. 0.6 ml of supernatants were incubated with or without lipo-

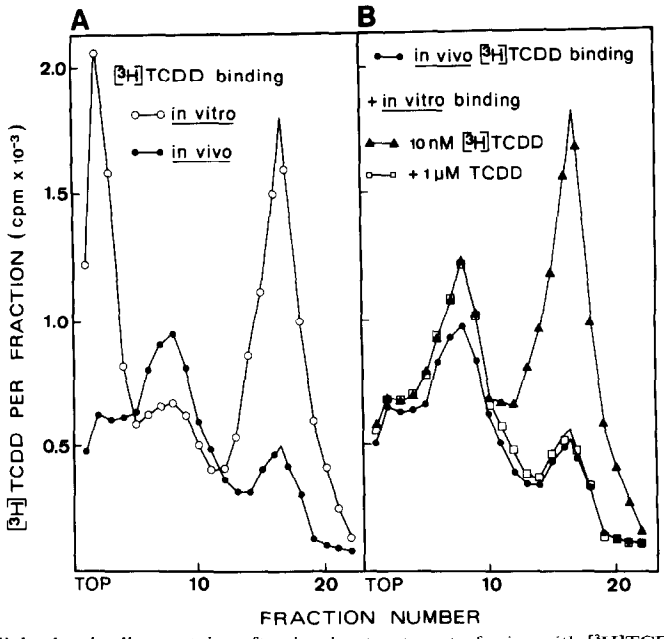

Fig. 2. Fate of light density lipoproteins after *in vivo* treatment of mice with [^{3}H]TCDD, analysed by sucrose gradient ultracentrifugation. (A) *in vivo* binding: [^{3}H]TCDD was injected intraperitoneally (3.6 μg/mouse = 0.570 μmol/kg body wt) to three B6 mice. Two hours later, livers were removed, homogenized in two volumes of HEDG and cytosol was prepared. Following dextran-coated charcoal treatment, 300 μl cytosol (5 mg protein/ml) was layered onto sucrose density gradient (5–20%) and examined as described in Materials and Methods (●); *in vitro* binding: B6 mouse hepatic cytosol (5 mg protein/ml) was incubated with 10 nM [^{3}H]TCDD, for 1 hr at 4°. Following dextran-coated charcoal adsorption, the sample was examined on sucrose density gradient as described in Materials and Methods (○). (B) *in vitro* binding of [^{3}H]TCDD (▲) following *in vivo* binding (●): one ml of cytosol (5 mg protein) from mice treated with [^{3}H]TCDD (see above) was incubated with 10 nM [^{3}H]TCDD, for 1 hr at 4°, then treated with dextran-coated charcoal and examined in sucrose gradient. Competition with [^{3}H]TCDD bound *in vivo* then *in vitro* was carried out by incubating (1 hr at 4°) 1 ml of cytosol (5 mg protein) from *in vivo*-treated mice with 10 nM [^{3}H]TCDD in presence of 1 μM non-labeled TCDD. Following dextran-coated charcoal adsorption the sample was examined in sucrose density gradient (□).

protein lipase (0.2 unit/ml) for 1 hr at 37°. Separated *Ah* receptor or 4S protein (2.5 mg protein in 0.6 ml) were added to the samples and incubated for 30 min at 0–4°. After removal of unbound radioligands, possibly released in the medium during the transfer step, aliquots (300 μl) were layered onto linear sucrose density gradients (5–20%) prepared in HEDG buffer. Gradients (4.8 ml) were centrifuged at 4° for 2 hr at 63,000 rpm in a Beckman VTi-65 vertical rotor. The radioactivity of the 22 fractions collected from each gradient was determined as described above.

RESULTS AND DISCUSSION

Processing of lipoproteins after treatment of mice with unlabeled TCDBF

Hepatic cytosols of untreated or 2,3,7,8 tetrachlorodibenzofuran-treated B6 mice have been examined, following Sudan black staining and [^{3}H]TCDD labeling, by single vertical spin potassium bromide density gradient ultracentrifugation. In these *in vivo* experiments, the aim of which is to study the lipoprotein processing subsequent to a xenobiotic administration, the mice were pretreated for different times (from 30 min to 30 hr) with various doses (from 10 μg to 1000 μg/mouse) of TCDBF. Figure 1 shows the qualitative as well as the quantitative changes in the lipoprotein patterns when B6 mice were pre-treated with 1000 μg/kg body weight for 1 hr, 16 hr and 30 hr. We observed, as early as 1 hr after treatment, a dramatic decrease of VLDL and LDL, which are then transformed to heavier entities, and, 16 hr after the treatment, an almost complete disappearance of LDL. Following this degradative phase, it is noteworthy that, at time 30 hr the pattern of lipoproteins was again similar to that existing in the cytosol of untreated mice. In control mice, only pretreated during the same periods of time with the solvent, any change was observed, by comparison with untreated mice, in the position and the amount of the different classes of lipoproteins. The alterations observed in TCDBF-treated mice were concomitant with an increase in radioactivity recovered in the bottom of the gradients, which contains the highest density material. This latter observation is reminiscent of that previously described for *in vitro* experiments [10], showing that lipoprotein lipase was able to convert, after 30 sec incubation at 37°, light lipoproteins (LDC) to heavier degradation products, some of them sedimenting to the bottom of sucrose gradients. These degradative effects of lipoprotein lipase have been investigated again in this work on both rat LDC and B6 mouse cytosol incubated for 30 sec or 5 min at 37° with the

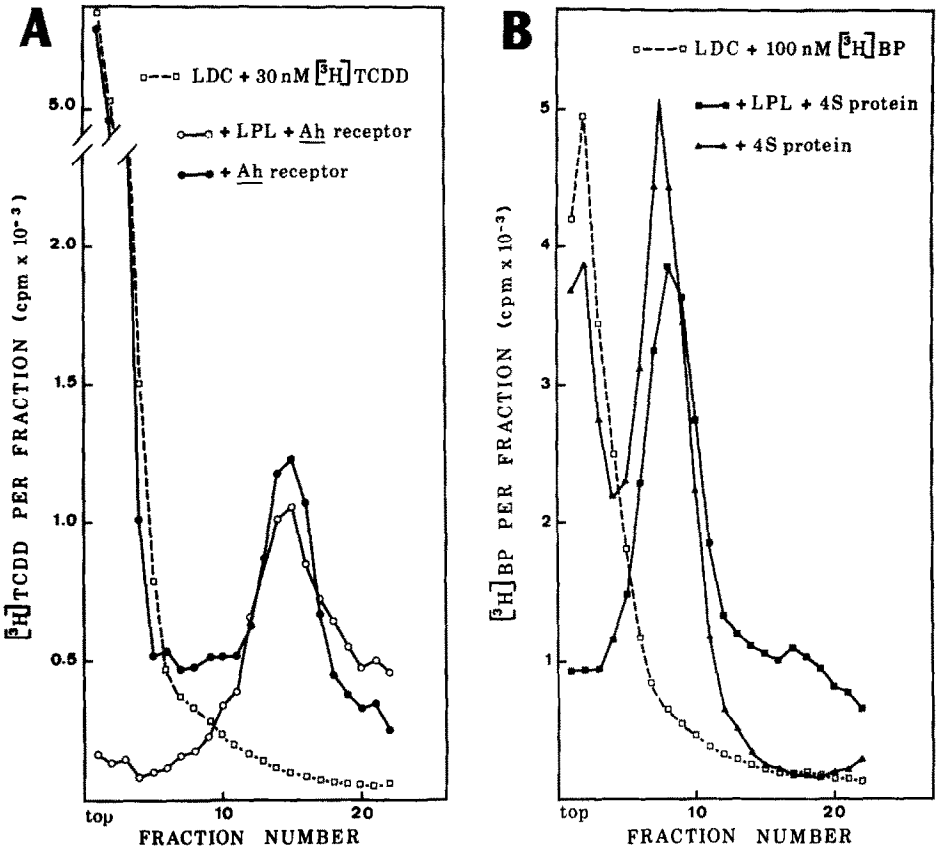

Fig. 3. *In vitro* transfer of [^{3}H]TCDD or [^{3}H]BP from lipoproteins to *Ah* receptor or 4S protein. (A) A 2 ml sample of light density component (LDC), separated from liver cytosol of B6 mice, was incubated with 30 nM [^{3}H]TCDD for 30 min at 4°. Following dextran-coated charcoal adsorption, 0.4 ml aliquot was kept in the ice for binding control when two other 0.6 ml aliquots were incubated with, or without, lipoprotein lipase (LPL) (0.2 unit/ml) for 1 hr at 37°. Separated *Ah* receptor (2.5 mg in 0.6 ml) was added to the samples and incubated for 30 min at 4°. After treatment with dextran-coated charcoal, aliquots (300 μl) of samples as well as [^{3}H]TCDD-LDC binding control were examined in sucrose density gradient as detailed in Materials and Methods. (B) The same procedure was used for the binding of 100 nM [^{3}H]BP to LDC sample and the transfer of [^{3}H]BP to the separated 4S protein.

enzyme; the analysis by vertical rotor sedimentation in KBr gradients showed that the various classes of Sudan black-stained lipoproteins were converted to heavy degradation products recovered as a blue-line in the bottom of the centrifuge tube (not illustrated). The kinetics of the density changes of LDL according to the different doses of TCDBF administered to mice has been investigated. It appears that both the extent and the duration of the LDL density changes are strongly dependent from the dose of TCDBF administered; the higher the TCDBF dose the longer the time is before return to a normal state occurs; this latter was observed after only 1 hr for 10 μg/kg TCDBF and 4 hr for 50 μg/kg TCDBF (not illustrated).

Fate of light density lipoproteins after in vivo *treatment of mice and rats with labeled TCDD.* Two hours after an *in vivo* administration of [^{3}H]TCDD to B6 mice (180 μg/kg) the binding of the radioligand to the cytosolic components of liver was, unexpectedly, observed primarily in the 4–5S region of the sucrose gradient (Fig. 2A). [^{3}H]TCDD was also bound to the *Ah* receptor, but modestly. In contrast with the *in vitro* situation (cytosol from untreated mice incubated with 10 nM [^{3}H]TCDD, also shown in Fig. 2A), very little radioactivity was detected in the top of the gradient after *in vivo* [^{3}H]TCDD treatment. As shown Fig. 2B, the further *in vitro* incubation of the cytosol from [^{3}H]TCDD-treated mice with [^{3}H]TCDD (10 nM), revealed that some additional binding occurred in the 4–5S region but, surprisingly, no [^{3}H]TCDD binding occurred in the top region of the gradient which usually contains the light density lipoproteins (see Fig. 2A, *in vitro* binding). Similar observations have been made with Sprague–Dawley rats receiving 90 μg/kg [^{3}H]TCDD (data not shown). In accordance with the data reported above, (Fig. 1), the light density lipoprotein–[^{3}H]TCDD complexes formed *in vivo* probably undergo some structural processing to heavier entities which are found, in the experiment shown in Fig. 2, in the 4–5S region of sucrose gradient. As previously demonstrated the high-density lipoproteins usually sediment in this part on the sucrose gradients [8]. The absence of [^{3}H]TCDD in the upper part of the gradient and, in contrast, its presence in the 4–5S region (high-density

lipoproteins) have also been observed, by this method, when [^{3}H]TCDD was incubated *in vitro* with the hepatic cytosol of B6 mice pretreated by either 100 μg/kg of TCDBF or 200 mg/kg 3-methylcholanthrene (not illustrated). Taken together the whole set of data shown in this study, supports the idea that when the intracellular lipoproteins were complexed *in vivo* with halogenated aromatic hydrocarbons they undergo a rapid and pronounced degradative processing (possibly catalysed by lipoprotein lipase) to heavier entities.

Regarding the *Ah* receptor, 2 hours after the [^{3}H]TCDD treatment, most (~ 90%) of this binding component was still unoccupied *in vivo*, and was able to bind the radioligand *in vitro* (Fig. 2B). It can be noted that subsequent *in vitro* incubation with a large excess of unlabeled TCDD was able to displace the [^{3}H]TCDD bound *in vitro*, whereas that bound *in vivo* to the *Ah* receptor was not displaced. It may be hypothesized that, in the *in vivo* induction process, the ligand binding triggers a structural change of the receptor leading to the sequestration of the [^{3}H]TCDD to the ligand-binding unit. This step would precede another major event of the receptor activation, namely the release of the 90-kDa heat shock protein, a subunit of the non-activated *Ah* receptor, [13, 14], since the sedimentation characteristic of the receptor is not modified (Fig. 2B).

In vitro *transfer of* [^{3}H]TCDD *and* [^{3}H]BP *from lipoproteins to* Ah *receptor or* 4S *protein*

Following the demonstration of a carrier-role of plasma lipoproteins for TCDD [1, 2] and BP [3–5] it was obvious that intracellular lipoproteins would be able to ensure a similar function; indeed, if polycyclic hydrocarbons, due to their hydrophobic features, easily pass through the cellular membranes [4] it can be presumed that the more hydrophilic environment of the cytosol would require the involvement of hydrophobic carriers. In order to test this possibility we have incubated cytosolic lipoproteins (LDC) with either 30 nM [^{3}H]TCDD or 100 nM [^{3}H]BP. Following charcoal–dextran removal of unbound hydrocarbons, the radioligand–lipoprotein complexes were incubated, after treatment with/without lipoprotein lipase, with either separated *Ah* receptor or 4S protein, respectively. It can be seen in Fig. 3. that the transfer of [^{3}H]TCDD to the *Ah* receptor (Fig. 3A) and that of [^{3}H]BP to the 4S protein (Fig. 3B) occurred from native as well as from lipoprotein lipase-disrupted lipoproteins. It seems likely, in spite of the lack of detailed quantitative studies in various conditions, that this passive exchange was dependent on both the relative affinities of the ligands towards the different cellular binding components as well as their quantitative binding capacity, as they are known according to the literature; this assumption was well illustrated (Fig. 4) by the complete transfer of [^{3}H]BP from the *Ah* receptor to the 4S protein, which displays a high affinity and a high binding capacity for this polycyclic aromatic hydrocarbon [10, 15–18]. Although the transfer of TCDD and BP occurs under the *in vitro* conditions described in this study, the *in vitro* findings do not necessarily mean that such transfers occur, to a significant extent, in the intact cell *in vivo*.

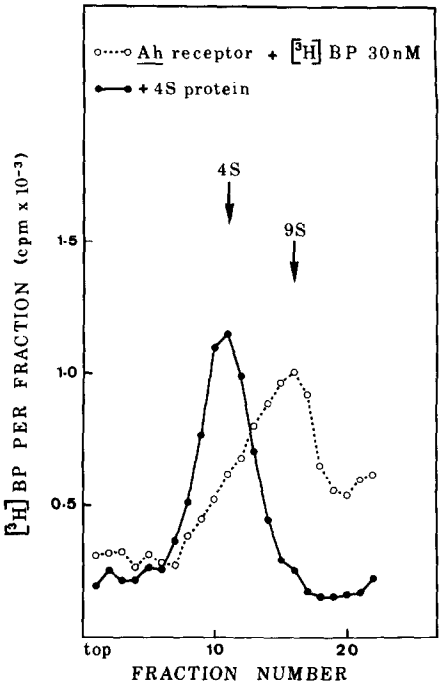

Fig. 4. *In vitro* transfer of [^{3}H]BP from the *Ah* receptor to the 4S protein. A 1 ml sample of *Ah* receptor (4.2 mg protein), separated from B6 mouse liver cytosol as described in Materials and Methods, was incubated with 30 mM [^{3}H]BP for 30 min at 4°. Following dextran-coated charcoal adsorption, a 0.4 ml aliquot was kept in the ice for [^{3}H]BP–*Ah* receptor binding control when 0.3 ml aliquot was incubated with 0.3 ml of separated 4S protein (1.25 mg protein) for 30 min at 4°. 300 μl aliquots of sample (●) or [^{3}H]BP–*Ah* receptor binding control (○) were examined on sucrose density gradients as detailed in Materials and Methods.

In conclusion, the present study shed some light on the role played by the lipoproteins in the transport and the biodistribution, at the molecular level, of lipophilic xenobiotics in the living organisms. After the pioneering work of Marinovitch *et al.* [1] showing that the binding of TCDD to plasma lipoproteins may delay toxicity in experimental hyperlipidemia, the exciting possibility now exists of accurately assessing the contribution of lipoprotein levels in various animal species, and especially in man, to the modulation of the toxicity of lipophilic xenobiotics. In connection with this it would be also of a prime interest to examine the effect of widespread drugs which affect lipoprotein production such as hypolipidemic agents (clofibrate, nicotinic acid) or lipoprotein catabolism (cholestyramine, D-thyroxine, etc) to the pharmacological action of drugs or the toxicological effect of pollutants.

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