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SEPARATION OF THE DIFFERENT CLASSES OF INTRAHEPATIC LIPOPROTEINS FROM VARIOUS ANIMAL SPECIES

THEIR BINDING WITH 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN AND BENZO(*a*)PYRENE

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Abstract—Using several analytical methods, including sucrose density gradient and potassium bromide density gradient ultracentrifugations, we have demonstrated that liver cells contain a range of lipoproteins somewhat distinct from those found in plasma. In addition to very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), many heavier entities have been found in the cytosol of various animal species. These heavier entities might represent either anabolic or catabolic intermediates of lipoproteins. Labelled hydrophobic xenobiotics such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or benzo(*a*)pyrene strongly bind to the various classes of lipoproteins and may be used as radioactive tracers in the analysis and possibly in the metabolic studies of intracellular lipoproteins. Moreover, this binding may be a prerequisite for a storage or/and a carrier—roles of lipoproteins in the intracellular distribution of lipophilic xenobiotics within the cells.

Lipoproteins are macromolecular aggregates consisting of a core of apolar (triglycerides and cholesteryl esters) lipids surrounded by polar (phospholipids and free cholesterol) lipids including specific apoproteins. Lipoproteins are the primary carriers of cholesterol in the blood and intensive studies were focused on plasma lipoproteins when a strong positive correlation between serum cholesterol levels and the incidence of atherosclerosis was found.

It is known that hepatocytes are the site of a shuttle-movement of lipoproteins which are either taken up by receptor-mediated endocytosis (LDL)† [1] or secreted by the smooth endoplasmic reticulum and the Golgi apparatus (VLDL) [2]. Various different particles or vesicles containing lipoproteins have been characterized within these cells [3, 4] but, in contrast with plasma lipoproteins, less is known about the characteristics and functions of intracellular lipoproteins. Recently, we have demonstrated, by incubating cytosol from rat and mouse liver with either [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), [^{3}H]3-methylcholanthrene or [^{3}H]benzo(*a*)pyrene (BP) that these compounds are able to bind to a light density component (LCD), the subsequent complex floating in the upper part of sucrose density gradients after ultracentrifugation [5]. In the same work this component was characterized as a mixture of light lipoproteins (VLDL and LDL).

In this report, we examine the comparative ability of various methods to separate and to analyse intracellular lipoproteins and we present data showing that (a) in addition to VLDL, LDL and HDL, heavier lipoprotein moieties are found in the liver of various animal species (mouse, rabbit, guinea-pig and hamster) and (b) that hydrophobic xenobiotics such as TCDD and benzo(*a*)pyrene bind to these different classes of lipoproteins and can be used as radioactive tracers in the metabolic studies of lipoproteins. Conversely, study of the fate of intracellular lipoproteins after *in vivo* administration of hydrophobic xenobiotics to animals, as developed in the companion paper [6], might help to better understand the role of intracellular lipoproteins towards these chemical compounds.

MATERIALS AND METHODS

Chemicals. Unlabelled 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 [7]. [G-^{3}H]benzo(*a*)pyrene (50.5 Ci/mmol), radiochemical purity 96.1% was purchased from Amersham (Bucks, England). Nonlabeled benzo(*a*)pyrene, Hepes, dextran (M_r 150,000), activated charcoal, VLDL, LDL and HDL isolated from human

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† Abbreviations used: LDL, low-density lipoproteins; VLDL, very low-density lipoproteins; HDL, high-density lipoproteins; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; BP, benzo(*a*)pyrene; LDC, light density component; TCPOBOP, 1,4-bis [2-(3,5-dichloropyridyloxy)]-benzene; Hepes, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; VHDC, very high-density component; B6, C57BL/6N Cr/BR micc; KBr, potassium bromide.

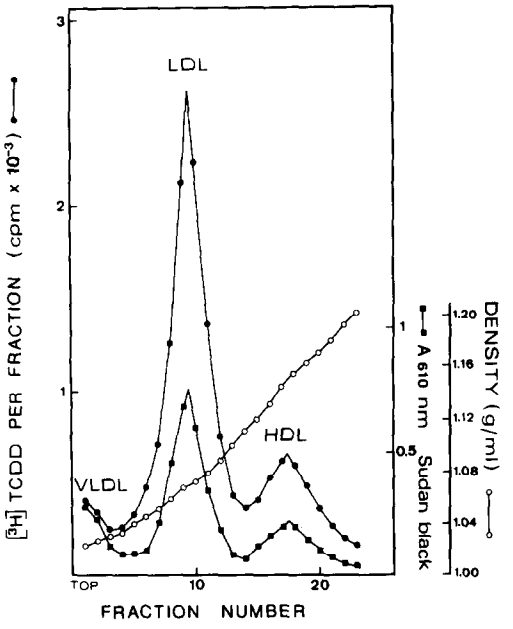

Fig. 1. Single vertical spin profile of plasma lipoprotein species after inverted rate-zonal ultracentrifugation. A mixture of VLDL, LDL and HDL isolated from human plasma (50 μl each) was incubated with 10 nM [^{3}H]TCDD, for 30 min at 4°, in 150 μl HEDG. Following dextran-coated charcoal adsorption, the supernatant was prestained by 150 μl Sudan black solution for 30 min at 4° in the dark. In order to determine the position of each lipoprotein species in the gradient, 50 μl samples of VLDL, LDL or HDL were treated by radioligand and Sudan black in the same conditions. Density of various samples was adjusted to 1.30 g/ml by the addition of solid KBr (550 mg in 1.75 ml). Aliquots (1.5-ml) were loaded in the bottom of 5 ml-tubes, and a discontinuous KBr density gradient was formed by overlayering 3.5 ml of 0.9% NaCl solution. Tubes were placed in a vertical rotor immediately after formation of the gradient and centrifuged at 4° for 105 min, at 63,000 rpm. Then 23 fractions were collected from each gradient. Radioactivity (●) and absorbance at 610 nm (■) were determined as described under Materials and Methods. Distribution of density (g/ml) in the fractions (○) was determined by refractometry.

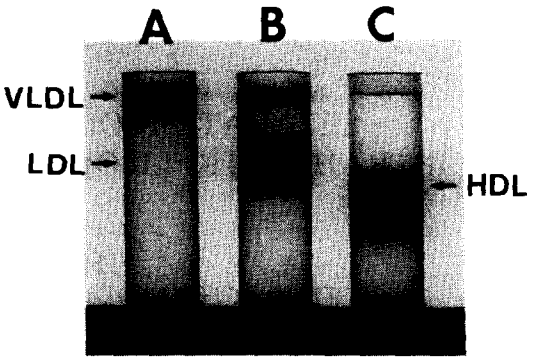

Fig. 2. Potassium bromide density gradient ultracentrifugation of lipoproteins in a swinging bucket rotor. 50 μl samples of VLDL (A), 10% VLDL plus 90% LDL (B) and HDL (C) were prestained by 100 μl Sudan black for 30 min at 4° in the dark. Following dilution in 300 μl HEDG the density of samples was adjusted to 1.30 g/ml by the addition of solid KBr (141.5 mg). 0.4 ml aliquots were loaded in the bottom of 5 ml tubes and four stock KBr density solutions were layered into the tubes as described in Materials and Methods. Tubes were then placed in a SW50.1 rotor immediately after formation of the gradient and centrifuged at 4° for 16 hr at 45,000 rpm.

plasma were obtained from Sigma (St Louis, MO). Dithiothreitol and dimethyl sulfoxide were purchased from Merck A.G. (Darmstadt, F.R.G.). Sudan black B was obtained from Aldrich (Milwaukee, WI). 1,4-Bis [2(3,5-dichloropyridyloxy)]-benzene (TCPOBOP) was a gift of Dr A. Poland (Madison, WI).

Animals. The animals were purchased from the following sources: C57 BL/6NCr/BR from Charles River, Cléon, France; tricolor guinea-pigs and New Zealand white rabbit from Cob-Labo-Lap, Yffiniac, France; Syrian Golden hamsters from Fichot, Ormesson, France. When the mice (20 g) were pretreated by TCPOBOP they received, by intraperitoneal route, 3 mg/kg of compound dissolved in sunflower oil and they were killed 72 hr after treatment. As described previously, treatment of rats and mice with phenobarbital [8] or mice with TCPOBOP, a phenobarbital-like inducer, increases the hepatic levels of binding components including lipoproteins and improves their study [5].

Buffers and solutions. The standard buffer used for the preparation of cytosols, the preparation of intracellular lipoproteins and the binding experiments was HEDG: 25 mM Hepes, 1.5 mM EDTA, 1 mM dithiothreitol and 10% glycerol (v/v), pH 7.6. Standard human plasma lipoproteins were dissolved in 10 mM Tris, 150 mM NaCl, 0.25 mM EDTA buffer pH 7.4. 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 and rabbit were killed by cervical dislocation whereas guinea-pigs and hamsters 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.* [9] 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), except for the rabbit, usually contained 20–25 mg of protein/ml. They were

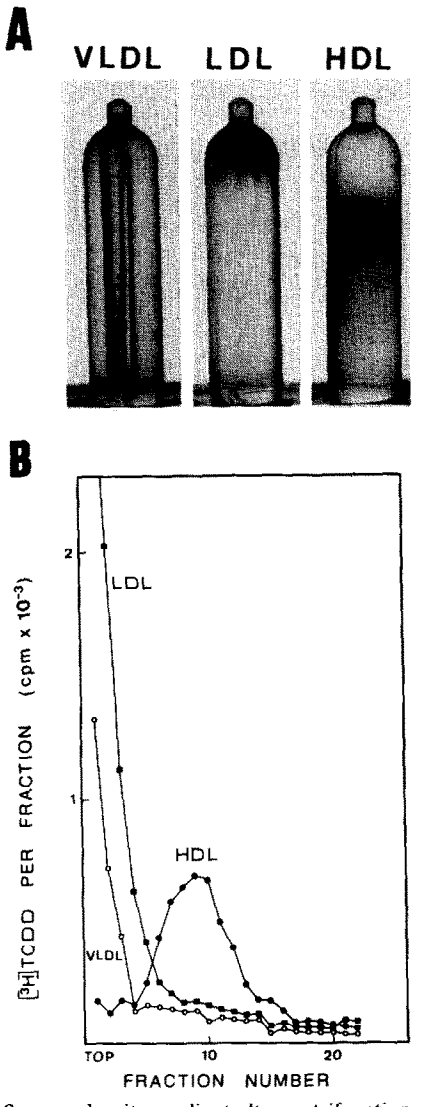

Fig. 3. Sucrose density gradient ultracentrifugation of various lipoprotein species. 50 μl samples of VLDL, LDL or HDL from human plasma were incubated with 10 nM [^{3}H]TCDD, for 30 min at 4°, in 250 μl HEDG. Following dextran-coated charcoal adsorption the supernatant was incubated with 100 μl Sudan black solution for 30 min at 4°. 300 μl aliquots of samples were layered onto linear sucrose density gradients (5–20%) prepared in HEDG buffer. Gradients (4.8 ml) were centrifuged at 4° for 2 hr in a vertical tube rotor at 63,000 rpm. After centrifugation the gradients were photographed (A) then twenty-two fractions (232 μl) were collected with a Beckman recovery system. Radioactivity in each fraction was determined by liquid scintillation counting (B).

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 TCPOBOP-treated B6 mouse liver 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 and 10 nM [^{3}H]benzo(*a*)pyrene 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). 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 [5], fractions 1 to 4 contain a mixture of light lipoproteins (VLDL and LDL), named LDC (light density component), fractions 6–12 contain the 4S protein and fractions 15–22 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 [5], 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. Light density lipoproteins (LDL, VLDL) can be roughly separated from high density lipoproteins (HDL) and analysed (after pre-staining or/and pre-labeling) by sucrose gradient centrifugation as described above. Two other methods used for serum lipoproteins have been adapted to intracellular lipoproteins. First, potassium bromide (KBr) density gradient ultracentrifugation in a vertical rotor as described by Chung *et al.* [10] was used: cytosol or lipoprotein samples were incubated with radioligands and/or Sudan black solution under various conditions (see figure legends), 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 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 formation 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

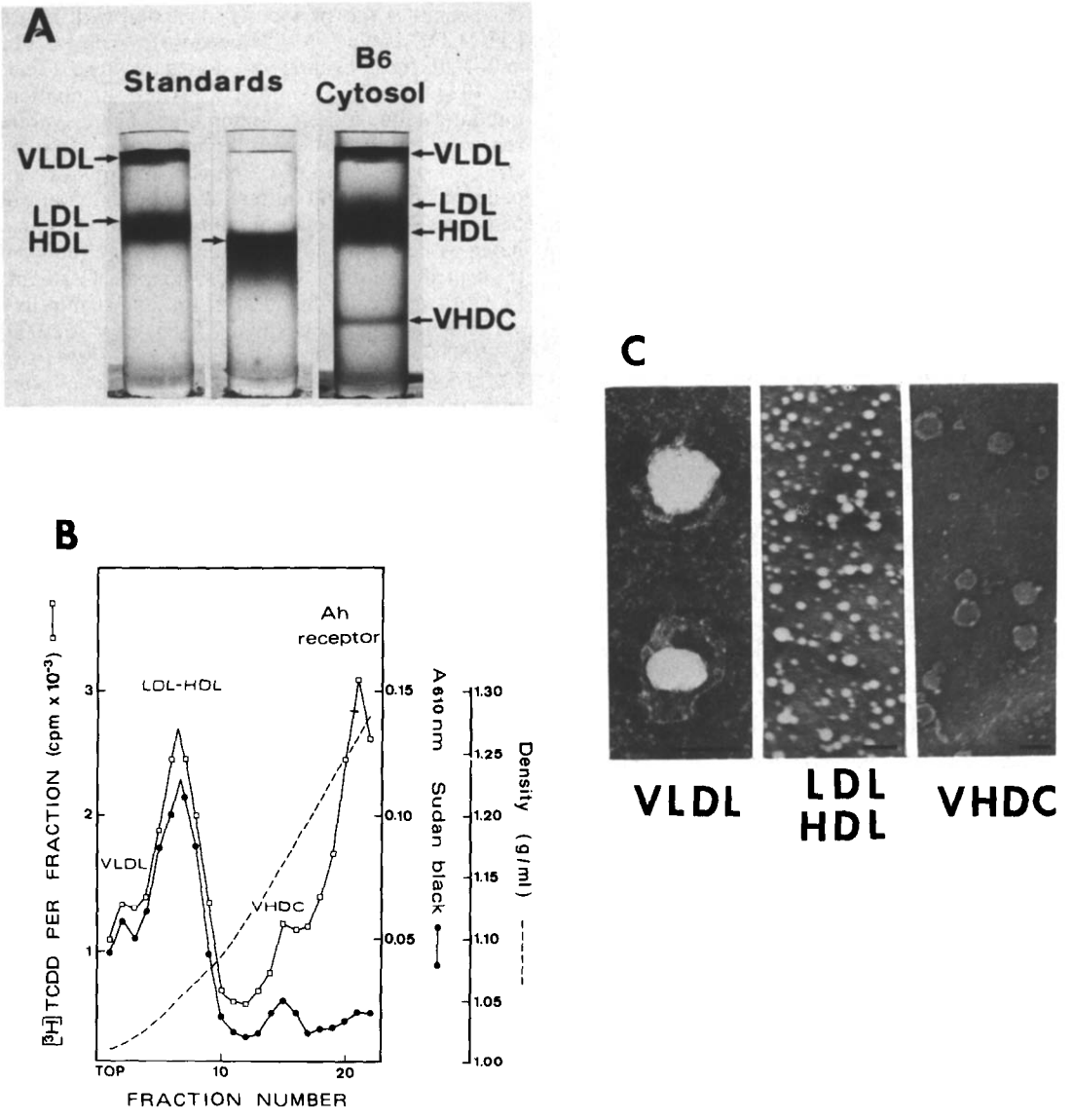

Fig. 4. Analysis of lipoproteins from mouse liver control by isopycnic potassium bromide density gradient ultracentrifugation. 0.5 ml of cytosol from C57 BL/6 mouse liver was incubated with 10 mM [^{3}H]TCDD for 1 hr at 4°. Following dextran-coated charcoal adsorption the supernatant (350 μl) was prestained with 100 μl Sudan black solution for 30 min at 4° in the dark. Standard human plasma lipoproteins (50 μl) were prestained in HEDG (300 μl) in the same conditions. Then the density of samples was adjusted to 1.30 g/ml by the addition of solid KBr (141.5 mg). 0.4 ml aliquots were loaded in the bottom of 5 ml tubes and four stock KBr density solutions were layered into the tubes as described in Materials and Methods. Tubes were then placed in a SW50.1 rotor immediately after formation of the gradient and centrifuged at 4° for 16 hr at 45,000 rpm. A, Partial separation of cytosolic lipoprotein species. B, Lipoprotein (●) and lipoprotein plus *Ah* receptor (□) profiles of mouse liver cytosol. The *Ah* receptor has been identified, in a separate ultracentrifugation experiment, by incubating a partially purified *Ah* receptor preparation (Ref. [5] with its specific ligand [^{3}H]TCDD (10 nM), in the absence or presence of a 100-fold molar excess of nonradioactive TCDD. C, Electron microscopy of separated lipoprotein species by pooling fractions 1-3(VLDL), 5-9(LDL + HDL) and 14-16(VHDC). Magnifications: VLDL, ×122,000; LDL, HDL, VHDC, ×54,000; bars = 100 nm.

stained lipoprotein bands was monitored spectrophotometrically at 610 nm. Second, isopycnic (equilibrium) density gradient ultracentrifugation in a swinging bucket rotor described by Kelley and Kruski [11] was employed: pre-labeled (or not) cytosol or lipoprotein samples (350 μl) were stained in the dark for 30 min with 100 μl of Sudan black solution. The density of samples (450 μl) was adjusted to 1.30 g/ml by the addition of solid KBr (141.5 mg); then 0.4 ml aliquots were loaded in the bottom of

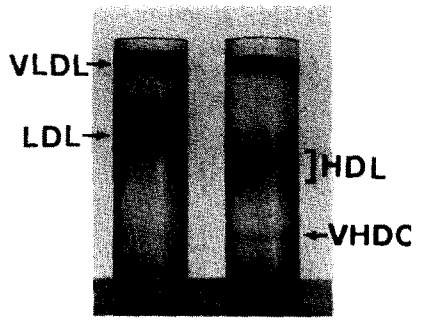

Fig. 5. Separation and analysis of light density lipoproteins (VLDL and LDL) and high density lipoproteins (HDL and VHDC) from mouse liver cytosol. Eight undiluted liver cytosol samples from TCPOBOP-treated C57 BL/6 mice (300 μl, 7.5 mg of protein) were layered on 5–20% sucrose density gradients and centrifuged at 63,000 rpm in a vertical tube VTI-65 rotor for 2 hr at 4°. Twenty-two fractions were collected then fractions 1–4 containing the light density lipoproteins and fractions 5-13 containing the high density lipoproteins (see Fig. 3B) were pooled then concentrated to 1.3 ml as described in Materials and Methods. 350 μl aliquots of separated samples were prestained with 100 μl Sudan black solution for 30 min at 4°. The density was adjusted to 1.30 g/ml by the addition of solid KBr (141.5 mg). The 0.4 ml aliquots were loaded in the bottom of 5 ml tubes and four stock KBr density solutions were layered into the tubes as described in Materials and Methods. The gradients were centrifuged at 4° for 16 hr at 45,000 rpm.

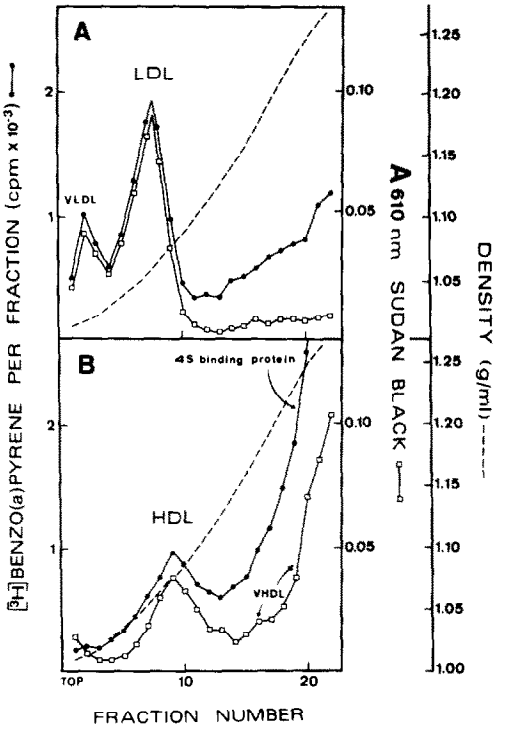

Fig. 6. Lipoprotein profiles and density of light density lipoproteins (A) and high density lipoproteins (B), from mouse liver cytosol, initially prepared by sucrose gradient ultracentrifugation then run individually on KBr density gradient. Three ml of undiluted liver cytosol from TCPOBOP-treated C57 BL/6 mice were incubated with 30 nM [^{3}H]benzo(a)pyrene for 1 hr at 4°. Following dextran-coated charcoal adsorption the supernatant was used to prepare, by sucrose gradient ultracentrifugation, each major density classe of lipoproteins. After concentration and Sudan black staining, the separated samples were examined in isopycnic KBr density gradient as described Fig. 5. Radioactivity (●), absorbance at 610 nm (□) and density were determined as described in Materials and Methods. The identification of the 4S protein in the bottom of the gradient was carried out, in a separate ultracentrifugation experiment, by incubating a partially purified 4S protein preparation (Ref. 5) with its specific ligand [^{3}H]BP (10 nM) in the absence or presence of a 100-fold molar excess of nonradioactive BP.

5 ml tubes and four stock KBr density solutions (checked using a density meter) were manually layered into the centrifuge tube as follows: 1.2 ml (density, 1.210 g/ml), 1.5 ml (density, 1.063 g/ml), 1.3 ml (density, 1.019 g/ml) and 0.5 ml (density, 1.006 g/ml). Tubes were placed in a SW50.1 rotor (Beckman) immediately after formation of the gradient and centrifuged at 4° for 16 hr at 45,000 rpm. After centrifugation, 22 fractions (eight drops per fraction) were collected. 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, separated *Ah* receptor and 4S protein or lipoprotein samples (1 ml) were incubated with [^{3}H]TCDD or [^{3}H]benzo(*a*)pyrene for 1 hr at 0–4°. The radioligands were added in 10 μl dimethyl sulfoxide; dimethyl sulfoxide also was used as the solvent for nonradioactive compounds 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.

Electron microscopy. Lipoprotein samples were examined by a negative staining technique using 2% phosphotungstate solution.

RESULTS AND DISCUSSION

Comparative ability of various methods for separation and analysis of lipoproteins

Serum lipoproteins can be quickly separated either by isopycnic [11] or single vertical spin [10] potassium bromide density gradient ultracentrifugation. The latter leads, after 105 min centrifugation at 63,000 rpm in a vertical rotor, to a very effective separation of the three main lipoprotein classes (Fig. 1) but we have observed that a part of VLDL tends to adhere to the inner wall of the vertical tube. So, it is difficult to ascertain whether a quantitative recovery of this lipoprotein class can be reached when gradient fractions are collected. In contrast, this uncertainty does not exist when VLDL are centrifuged at 45,000 rpm for 16 hr in a swinging bucket rotor (Fig. 2). By this method, LDL are readily separated from VLDL but are much less efficiently separated from HDL.

As previously demonstrated [5], 5–20% sucrose gradient centrifugation in a vertical rotor allowed the separation of a mixture of intracellular light

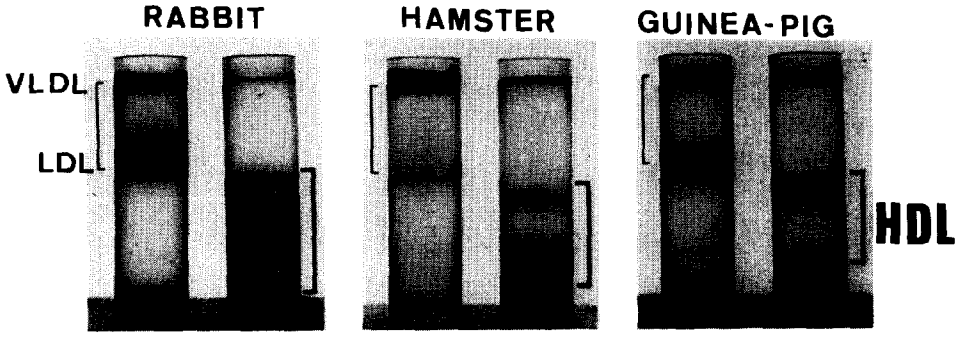

Fig. 7. Separation and analysis of lipoproteins from liver cytosol of rabbit, hamster and guinea-pig. The two major density classes of lipoproteins (VLDL, LDL) and (HDL, VHDC) were initially separated by sucrose gradient ultracentrifugation then examined in isopycnic KBr density gradient ultracentrifugation as described Fig. 5.

lipoproteins from other binding components (*Ah* receptor and 4S protein) of mouse or rat liver cytosol; this preparation, which we called LDC (light density component), contained VLDL and LDL. Nevertheless, as mentioned above and as shown in Fig. 3A, with human serum lipoproteins a certain percentage of VLDL adhere to the wall of the centrifuge tube. Under the same conditions, high density lipoproteins sediment in the 4–6 S region of the gradient (near fractions 5–13, (Fig. 3B). So, this method, in contrast to that using isopycnic KBr density gradient centrifugation (Fig. 2), should be a convenient means to separate the light density lipoproteins (VLDL and LDL) from high density lipoproteins (HDL) contained in the hepatic cytosol of mice and other animal species (see below). Interestingly, it can be noted that radioactive non-halogenated polycyclic aromatic hydrocarbons such as benzo(*a*)pyrene, as well as halogenated aromatic hydrocarbons, such as TCDD, bind to lipoproteins (Figs 1 and 3B) and can be used, in addition to Sudan black staining, as tracers of the different classes of lipoproteins. As demonstrated previously, these compounds bind to the phospholipid moiety of the lipoproteins on condition that their quaternary structure is not altered [5]. Control experiments, performed without labeled BP and TCDD, indicate that these compounds and/or their vehicle do not influence the profile of lipoproteins in the gradients (data not shown).

Analysis of lipoproteins in C57 BL/6 mouse liver cytosol

Figure 4A shows the partial separation of prestained and prelabelled lipoproteins from B6 mouse hepatic cytosol after centrifugation at 45,000 rpm for 16 hr in isopycnic KBr density gradient. As expected, VLDL were floating at the top of the gradient while LDL and HDL were found together in the upper half of the gradient. As can be seen in the lower half of the gradient, a very high density component (VHDC) exists in the hepatic cytosol and this is stained by Sudan black. Gradients were fractionated and Fig. 4B shows the radioactivity as well as the Sudan black profiles obtained. The latter corresponds to the lipoprotein components only while [^{3}H]TCDD radioactivity was found to the same peaks but also bound to the *Ah* receptor, which remained in the bottom of the KBr gradient after centrifugation, as demonstrated in a control experiment with separated *Ah* receptor (data not shown). The three groups of components (as shown Fig. 4A) from B6 cytosol were separated by pooling fractions 1–3 (VLDL), 5–9 (LDL + HDL) and 14–16 (unknown very high density component); they were concentrated (to 200 μl) by centrifugation at 3,000 rpm in Centriflo membrane cones CF25 (Amicon); the samples were examined by a negative staining technique in electron microscopy and it can be seen Fig. 4C that, in contrast to the white aspect of VLDL, LDL and HDL, the unknown very high density component (VHDC) consists only of dark particles, which appear as completely cleared out of the triglycerides that are normally localized in the core of the lipoprotein particles. It can be hypothesized that these atypical particles represent either triglyceride-poor nascent lipoproteins or products of lipoprotein catabolism by lipoprotein lipase.

It might be asked whether cytosolic preparations are unequivocally representative of the cell inside or, in other words, are devoided of blood serum contamination. To avoid such an eventuality, precautionary measures such as extensive perfusion and washing of livers have been taken. Moreover, control experiments, with blood serum itself, have been carried out, such as described in our previous report [5]; our conclusions, which exclude serum contamination, were in complete agreement with those of Denison *et al.* [12] who thoroughly studied this possible artefact, the probability of which was raised by Poellinger *et al.* [13].

Analysis of different classes of lipoprotein in the hepatic cytosol from various animal species

Following separation of light density lipoproteins (VLDL and LDL) from high density lipoproteins (HDL + VHDC) by sucrose gradient centrifugation, these two groups of lipoproteins contained in the hepatic cytosol from various animal species were concentrated and then analysed by isopycnic potassium bromide density gradient ultracentrifugation. Firstly, Fig. 5 shows that sucrose gradient centrifugation truly leads to a good separation of light density lipoproteins (VLDL + LDL) from a whole set of higher density lipoproteins of C57 BL/6 mouse

cytosol.

The pre-labeling as well as the pre-staining of the separated two major groups of lipoproteins before centrifugation in isopycnic KBr density gradient, were used to estimate the density of each class of lipoproteic components in B6 hepatic cytosol (Fig. 6). VLDL were floating in the top of the gradient ($d < 1.006$ g/ml) while LDL and HDL were recovered around fractions 7–8 (d_{av} 1.055 g/ml) and fraction 9 (d_{av} 1.075 g/ml) respectively. As for very high density component, it was found in fraction 16, the density of which was 1.180 g/ml. It should be noted (Fig. 6B) that a large amount of heavier components, stained by Sudan black, exist in the liver cytosol (fractions 18–22). In addition to the latter, the binding proteins *Ah* receptor (Fig. 4B) or 4S protein (Fig. 6) remain in the bottom of KBr density gradients after centrifugation as checked with separated samples incubated with radioligands in presence or absence of a 100-fold molar excess of nonradioactive competitor (not illustrated).

Making use of the procedure of sucrose density followed by KBr gradient centrifugations, we analysed the lipoproteins in hepatic cytosol of rabbit, hamster and guinea-pig. It can be seen Fig. 7 that beside the typical VLDL, LDL and HDL families, the cytosol of these three animal species contained, compared to that of the B6 mouse, more (rabbit, hamster) or less (guinea-pig) heterogeneous heavier population of lipoproteins.

From a perspective of toxicology, the binding of hydrophobic chemicals such as TCDD and BP to the different classes of lipoproteins raises the question of an eventual role of these lipid components in the cells. The storage, or/and the transport or the intracellular distribution of lipophilic xenobiotics to various organelles (nucleus, mitochondria, smooth endoplasmic reticulum) could be ensured by lipoproteins. On the other hand, it may be asked whether the lipoproteins display a modulating action on the toxicological effects of chemicals. The data shown in the companion [6] paper bring some insights to a few of these different questions.

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