Previous results have demonstrated that liver membranes possess two distinct lipoprotein receptors: a low density lipoprotein (LDL) receptor that binds lipoproteins containing either apolipoprotein (apo-) B or apo-E, and an apo-E-specific receptor that binds apo-E-containing lipoproteins, but not the apo-B-containing LDL. This study reports the isolation and purification of apo-B,E(LDL) and apo-E receptors from canine and human liver membranes. The receptors were solubilized with the zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate and were partially purified by DEAE-cellulose chromatography. The apo-B,E(LDL) receptor was isolated by affinity chromatography on LDL-Sepharose. The apo-E receptor, which did not bind to the LDL-Sepharose column, was then purified by using an HDL, (cholesterol-induced high density lipoprotein)-Sepharose affinity column and an immunaffinity column. Characterization of the receptors revealed that the hepatic apo-B,E(LDL) receptor is similar to the extrapathic LDL receptor with an apparent $M_r = 130,000$ on non-reducing sodium dodecyl sulfate-polyacrylamide gels. The apo-E receptor was found to be distinct from the apo-B,E(LDL) receptor, with an apparent $M_r = 56,000$. The purified apo-E receptor displayed Ca$^{2+}$-dependent binding to apo-E-containing lipoproteins and did not bind to LDL or chemically modified apo-E HDL, and HDL, in the absence of Ca$^{2+}$. Antibodies raised against the apo-B,E(LDL) receptor cross-reacted with the apo-E receptor. However, an antibody prepared against the apo-E receptor did not react with the apo-B,E(LDL) receptor. The apo-E receptor also differed from the apo-B,E(LDL) receptor in amino acid composition, indicating that the apo-E receptor and the apo-B,E(LDL) receptor are two distinct proteins. Immunoblot characterization with anti-apo-E receptor immunoglobulin G indicated that the apo-E receptor is present in the hepatic membranes of man, dogs, rats, and mice and is localized to the rat liver parenchymal cells.

The liver is the major organ in the body involved in the regulation of cholesterol metabolism. In addition to its role in lipoprotein biosynthesis and secretion (1), the liver is also responsible for the clearance of apo-B- and apo-E-containing lipoproteins (2). Several studies have indicated that the liver is responsible for >50% of the total body low density lipoprotein (LDL') catabolism in many different species (3-6). Results from studies comparing the tissue uptake of native and chemically modified LDL reveal that the liver uptake of LDL is mainly mediated by a receptor-dependent process (7, 8). Kovanen et al. (9), using an in vitro membrane binding assay, have demonstrated the presence of the LDL receptor on liver membranes. Our results have shown that the hepatic apo-B,E(LDL) receptor is similar to the extrapathic apo-B,E(LDL) receptor in its ability to interact with both apo-B-containing LDL and apo-E-containing lipoproteins (10).

A high affinity receptor-mediated process is also responsible for the hepatic uptake of chylomicron remnants (11). However, several lines of evidence have indicated that an additional lipoprotein receptor on liver membranes may be involved in chylomicron remnant uptake. First, chylomicron remnant catabolism is nearly normal in type II familial hypercholesterolemic patients who have defects in the apo-B,E(LDL) receptor pathway (12). Second, the Watanabe Heritable Hyperlipidemic rabbits, which express nearly normal chylomicron remnant uptake by the liver (14). In addition, animals fed a high cholesterol diet display a retarded clearance rate for LDL due to the suppression of apo-B,E(LDL) receptor activity (2). However, chylomicron remnant clearance in these cholesterol-fed animals is nearly normal (15). Taken together, these results suggest the presence of an additional hepatic receptor responsible for the uptake of chylomicron remnants.

The hepatic chylomicron remnant receptor appears to be specific for the E apolipoprotein. In the perfused rat liver, the rate of chylomicron remnant uptake resembles that observed for apo-E HDL, (the cholesterol-induced HDL containing only apo-E) (16). Competitive binding studies also indicate that both chylomicron remnants and apo-E HDL, are taken up by the liver by the same process (18). The apo-E HDL, have been shown to be taken up primarily by a receptor-mediated process, specifically by hepatic parenchymal cells (17). These studies, as well as others, have implicated the role of apo-E in mediating the hepatic uptake of chylomicron

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‡ Established Investigator of the American Heart Association. To whom correspondence and reprint requests should be addressed: Gladstone Foundation Laboratories, P. O. Box 40608, San Francisco, CA 94140-0608.

1 The abbreviations used are: LDL, low density lipoproteins; HDL, cholesterol-induced high density lipoproteins; CHAPS, 3-[apo, apolipoprotein; 3-cholamidopropyl]-dimethylammonio]-1-propanesulfonate; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; VLDL, very low density lipoproteins; apo-, apolipoprotein; Heps, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
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remnants (16–19). More recently, Arbeeny and Rifici (20) have shown that chylomicron remnants containing apo-E are removed by the rat liver at a slow rate. Furthermore, apo-E-deficient chylomicron remnants are not able to interact with receptors on dog liver membranes (21). The importance of apo-E in mediating chylomicron remnant clearance has been confirmed by studies in patients with familial type III hyperlipoproteinemia. The apo-E isolated from these patients (apo-E2) binds poorly to the liver receptors (22, 23) and does not promote hepatic uptake of apo-E2-phospholipid complexes (24).

An apo-E-specific receptor, which may represent the chylomicron remnant receptor, has been identified recently in liver membranes of dogs, humans, and swine (10, 25). This receptor, referred to as the apo-E receptor, is different from the apo-B,E(LDL) receptor in that it binds apo-E-containing lipoproteins, but does not interact with apo-B-containing LDL (10). In contrast to the apo-B,E(LDL) receptor, the apo-E receptor is not regulated by metabolic conditions (33). In this study, the apo-E receptors from canine and human liver membranes have been purified. Characterization of the apo-E receptor revealed that it is a distinct protein with a Mr = 58,000 and that it is not a cysteine-rich protein. A recent communication by Kinoshita et al. (34) describes the isolation of a Mr = 36,000 protein from the rat liver that is postulated to be the apo-E receptor. The relationship between this protein and the Mr = 58,000 apo-E receptor remains to be determined.

**EXPERIMENTAL PROCEDURE**

**Materials**—The Bolton-Hunter reagent, carrier-free 125I, and 125I-labeled anti-rabbit immunoglobulin G (IgG) were purchased from Amersham Corp. Egg lecithin was obtained from Avanti Polar Lipids (Birmingham, AL). Nucoll Tween acetate membrane filters (0.45-µm pore size) were purchased from Oxoid Ltd. (Basingstoke, England). Protein-A-Sepharose, Sephadex G-25M, and CNBr-activated Sepharose were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Sodium sucrum and Trasylol were obtained from FBA Pharmaceuticals (New York, NY). Iodo-beads and CHAPS were supplied by Pierce Chemical Co. Octyl-β-D-glucoside, phenylmethylsulfonyl fluoride (PMSF), and bovine serum albumin were purchased from Calbiochem-Behring. Leupeptin was obtained from Vega Biochemicals (Tucson, AZ). The anion exchange resin DE52 was purchased from Whatman Ltd. (Maidstone, England).

**Animals and Diet**—Adult foxhounds (>18 months of age) were purchased from Brink Farms (Paola, KS) and were maintained on either normal dog chow or a semi-synthetic diet containing 16% coconut oil and 5% cholesterol (35).

**Lipoprotein Preparation.**—Human LDL (d = 1.02–1.06 g/ml) from fasted donors were isolated by centrifugation at 18 h at 69,500 rpm in a 60 Ti rotor (Beckman Instruments, Palo Alto, CA). The human LDL were washed by recentrifugation at d = 1.06 g/ml for 16 h at 69,500 rpm. Dogs were fasted for 16 h before plasma lipoproteins were isolated. Canine LDL were isolated from the plasma of normolipidemic foxhounds by ultracentrifugation (d = 1.02–1.063 g/ml) at 59,000 rpm for 18 h and were purified by Pevikon block electrophoresis (36). The apo-E HDL (d = 1.06–1.02 g/ml) and HDLd (d = 1.02–1.063 g/ml) were isolated from the plasma of hypercholesterolemic dogs and were purified by Sep-Pak Pevikon electrophoresis (36). Dog chylomicron remnants and VLDL remnants (β-VLDL fractions I and II, respectively) were isolated from the plasma of cholesterol-fed animals as described (37).

**Chemical Modifications of Lipoproteins—**Cycloexenzanidine was used to modify the arginines residues of the lipoprotein as described (28). Two micrograms of lipoprotein protein in 1 ml of 0.15 M NaCl, 0.01% EDTA were mixed with 2 ml of 0.15 M 1,2-cycloexanediene in 0.2 g sodium borate (pH 8.1) for 2 h at 37°C. The lysine residues were modified by reductive methylation using formaldehyde and sodium borohydride as described previously (42). The chemically modified lipoproteins were dialyzed exhaustively against 0.15 M NaCl-EDTA before use. As previously demonstrated, cycloexenzanidine modification or reductive methylation abolished the ability of LDL and apo-E HDL to bind to the apo-B,E(LDL) receptor on fibroblasts (41, 42).

**Preparation of Affinity Columns**—Human LDL (d = 1.02–1.06 g/ml) and canine HDLd (d = 1.06–1.02 or 1.02–1.063 g/ml fractions; Pevikon block-purified), or rabbit anti-apo-E receptor IgG were coupled to CNBr-activated Sepharose in accordance with the manufacturer’s recommendations (43). A ratio of 30 mg of ligand protein to 1 g of dry gel was used. All affinity columns were stored in Buffer IV and were used within a week.

**Buffers**—Buffer I contained 50 mm Tris-HCl (pH 9.0), and 2 mm CaCl2; Buffer II contained 50 mm Tris-HCl (pH 9.0), 2 mm CaCl2, 100 mm NaCl, 60 mm CHAPS, and 60% glycerol (v/v); Buffer III contained equal volumes of Buffers I and II; Buffer IV contained 50 mm Tris-HCl (pH 9.0), 2 mm CaCl2, and 30% glycerol (v/v); and Buffer V was prepared by mixing 1 volume of Buffer II with 11 volumes of Buffer IV. All buffers contained 0.5 mm PMSF, 1 kU/ml Trypsin, 0.05 mm leupeptin, and 1 mm sodium azide. The solutions were sterilized by filtration through 0.45-µm pore size filters before use.

**Solubilization of Membrane Proteins—**Livers from normal (plasma cholesterol concentration = 100–150 mg/dl) or cholesterol-fed dogs (plasma cholesterol concentration = 500–700 mg/dl) were used in this study. The dogs were anesthetized with pentobarbital, and the livers were perfused in situ with ice-cold saline containing 1 mm PMSF and 1 kU/ml Trypsol. The livers were cut into this slices and put into cold 0.15 M NaCl containing 0.5 mm PMSF and 1 kU/ml Trypsol. Homogenate was obtained from a 47-year-old male from this preparation. The fraction containing the peak activities were pooled and applied without delay to a affinity column.

**Purification of the apo-E receptor**—was performed at 4°C. Membrane protein was solubilized in Buffer III at a concentration of 1 mg of membrane protein/ml of buffer (usually 500 mg of membrane were used in a typical study). The sample was mixed for 1 h, and the insoluble materials were pelleted by centrifugation for 1 h at 200,000 × g. The clear supernatant was applied immediately to a DE52-cellulose column.

**Data Analysis**—The detergent-solubilized sample was applied to a DE52-cellulose column (2.5 × 7 cm), previously equilibrated with Buffer III, at a flow rate of 150 ml/h. The column was washed with 100 ml of Buffer II, and the proteins that bound to the column were eluted with a 100-ml salt gradient composed of 50–300 mM NaCl in Buffer III. Samples were collected in 3-ml fractions, and the receptor activity in each fraction was determined as described above. The fraction containing the peak activities were pooled and applied without delay to a affinity column.

**Effect of dietary cholesterol on the expression of the apo-E receptor on liver membranes**—Dog chylomicron remnants and VLDL remnants (β-VLDL fractions I and II, respectively) were isolated from the plasma of cholesterol-fed animals as described (37). The isolated lipoproteins were dialyzed exhaustively against 0.15 M NaCl, 0.01% EDTA before use. As previously demonstrated, cycloexenzanidine modification or reductive methylation abolished the ability of LDL and apo-E HDL to bind to the apo-B,E(LDL) receptor on fibroblasts (41, 42).
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Sephadex G-25M column equilibrated with Buffer III. This procedure yielded proteins with a radioactivity of 50–200 cpm/pg protein.

Affinity Chromatography—The DEAE-purified proteins were applied to an LDL-Sepharose column (1 × 4 cm) to remove the apo-B,E(LDL) receptor. The sample was washed 7–10 times at a flow rate of 2–10 ml/h, and the column was then washed in 1 ml of Buffer III supplemented with 300 ml of Buffer II, 100 ml of Buffer V, and 100 ml of Buffer III. The apo-B,E(LDL) receptor that bound to the column was eluted at a flow rate of 10 ml/h with 20 mM sodium sulam in Buffer III or with 0.5 M NH₄OH as described (47, 48).

The LDL-Sepharose-unbound fraction, containing the apo-E receptor, was applied immediately to an HDL-Sepharose column (1 × 4 cm), and the sample was recycled through the column 10 times at a flow rate of 10 ml/h. The HDL-Sepharose column was then washed according to the procedure described above. Proteins that bound to the HDL-Sepharose column were eluted at a flow rate of 10 ml/h with 20 mM sodium sulam in Buffer III or with 0.5 M NH₄OH.

Binding Assays—The binding of lipoproteins to the liver membranes was determined using the method of Basu et al. (49). All assays were performed at 4°C in 100 μl of incubation buffer containing 50 mM Tris-HCl (pH 7.5), 25 mM NaCl, 1 mM CaCl₂, and 20 mg/ml bovine serum albumin. Each assay tube contained 100–200 μg of membrane protein, and the 125I-lipoproteins were added at concentrations indicated in the text. Nonspecific binding was determined by the inclusion of 20 mM EDTA in the assay tube.

The detergent-solubilized receptor was assayed by the phosphatidylcholine/acetone method of Schneider et al. (47). The CHAPS concentration in the solution was diluted to below 1 mM in the presence of 0.38 mg/ml phosphatidylcholine and 45.5% acetone. The precipitate was collected by centrifugation for 20 min at 20,000 rpm (Beckman JA 14 rotor). The receptor, complexed to the phosphatidylcholine/acetone fraction, was complexed to phosphatidylcholine and incubated for 4 h with 125I-labeled lipoproteins. Radiolabeled lipoproteins bound to the receptors were separated from the unbound lipoproteins by filtration on cellulose acetate membrane filters (47).

The stoichiometry of 125I-apo-E HDL₃ binding to the apo-E receptor was determined according to the method of Schneider et al. (58). The detergent-solubilized receptor, purified from the 125I-labeled DEAE fraction, was complexed to phosphatidylcholine and incubated with 2 μg/ml 125I-labeled or unlabeled apo-E HDL₃ for 1 h at 23°C. The receptor-phospholipid complex was then isolated by filtration on a cellulose acetate filter. The number of receptors on each filter was determined by the radioactivity trapped on the filter in the presence of unlabeled apo-E HDL₃. The radioactivity observed on the filter when the incubation was performed with 125I-apo-E HDL₃ was the sum of the radioactivity from both the apo-E receptor and the lipoprotein. The amount of 125I-apo-E HDL₃ bound to the receptor was then determined by subtracting the radioactivity due to the 125I-labeled HDL₃ from the total radioactivity on the filter.

The specific activity of the 125I-labeled apo-E receptor was determined by subtracting the radioactivity due to the 125I-labeled HDL₃ from the total radioactivity on the filter. Based on the specific activity of the 125I-labeled apo-E receptor and the 125I-apo-E HDL₃, the data could then be expressed as the amount of apo-E HDL₃ bound per μg of receptor protein. The Kd and Bmax were determined by Scott's analysis of direct binding data (50).

Preparation of Antibodies against the Apo-B,E(LDL) Receptor and Apo-E Receptor—Bovine apo-B,E(LDL) receptor was purified as described by Schneider et al. (28, 47). New Zealand White rabbits (2.5–3 kg) were injected subcutaneously with the purified apo-B,E(LDL) receptor. The antibody levels were determined using radioimmunoassay (RIA) as described by Schneider et al. (57). Antibody levels were determined using radioimmunoassay (RIA) as described.

The binding activity of the CHAPS-solubilized apo-E receptor was measured by diluting the detergent concentration to below 1 mM (critical micellar concentration of 9 mM), followed by precipitation of the solubilized proteins using the phosphatidylcholine/acetone method of Schneider et al. (47). The precipitated receptor was used in a solid phase assay to determine the binding of 125I-apo-E HDL₃. Results showed that the binding activity of the solubilized receptor was dependent on the detergent concentration and the pH of the buffer used to solubilize the liver membranes. The optimum conditions were 30 mM CHAPS (Fig. 1) at pH 7.4 (data not shown). Under these conditions, the 125I-apo-E HDL₃ wassolubilized by 30% glycerol, the solubilized receptor was stable for 1 week at 4°C. The receptor was inactive at high detergent concentrations (>60 mM) and was unstable at a pH lower than 9.0. Therefore, 30 mM CHAPS and pH 9.0 were used as the standard conditions to solubilize dog liver membranes for the purification of the apo-E receptor.
**Hepatic Apo-E (Remnant) Receptor**

4259

Octyl Glucoside

3

10 20 30 40 50 60

Detergent Concentration (mM)

FIG. 1. Solubilization of apo-B,E(LDL) and apo-E receptors from canine liver membranes. Canine liver membranes (100,000 x g pellet) in Buffer I were mixed for 1 h at 4 °C with an equal volume of Buffer II containing either CHAPS (●) or octyl glucoside (▲). The solubilized proteins were separated from the insoluble material by centrifugation for 1 h at 100,000 x g. The solubilized proteins were precipitated with acetone in the presence of phosphatidylcholine and tested for binding activity with 125I-apo-E HDL₄ (2 µg of protein/ml) as described under “Experimental Procedures.” The structure of CHAPS is shown in the inset.

3.5

3.0

2.5

2.0

1.5

1.0

0.5

1 5 10 15 20 25 30

Fraction Number

FIG. 2. DEAE-cellulose chromatography of solubilized canine liver membranes. Nine hundred milligrams of solubilized canine liver membranes in Buffer III were applied to the DEAE-cellulose column as described under “Experimental Procedures.” The unbound fraction was collected as a single fraction, and the column was eluted with a linear 100-ml salt gradient composed of 50-300 mM NaCl in Buffer III (top panel). Aliquots of each fraction were removed for protein determination (○) and for the binding assay with 2 µg of 125I-apo-E HDL₄ (●).

The first step in the purification of the solubilized apo-E receptor was anion exchange chromatography on DEAE-cellulose. When 900 mg of liver membrane protein from an adult dog were solubilized in Buffer III and applied to the DEAE-cellulose column, most of the protein in the extract was unbound and emerged from the column (Fig. 2). This DEAE-unbound fraction did not display detectable receptor binding activity for 125I-apo-E HDL₄. The proteins bound to the DEAE column were eluted with a 50–300 mM NaCl gradient, and the fractions were analyzed for 125I-apo-E HDL₄ binding activity (Fig. 2). The fraction containing the major activity eluted at about 100–120 mM NaCl. Greater than 90% of the original apo-E HDL₄ binding activity was present in these fractions. The DEAE column subfractions of the solubilized membrane proteins resulted in a 20- to 25-fold increase in apo-E HDL₄ binding activity per mg of protein when compared with the 100,000 x g membrane fraction.

When the DEAE-purified protein from adult dog liver membranes was tested for receptor binding activities with both LDL and apo-E HDL₄, significant binding activities for both lipoproteins were observed (Fig. 3A). The characteristics of LDL binding were very similar to LDL binding to the apo-B,E(LDL) receptors of extrahepatic cells. The binding was high affinity ($K_d = 7–20$ nM) and Ca²⁺-dependent. Furthermore, cyclohexane-modified LDL did not bind to the apo-B,E(LDL) receptor in the DEAE fraction. In addition, incubation of the DEAE fraction with 30 µg/ml Pronase for 1 h at 37 °C completely abolished its ability to bind LDL. These results demonstrated that dog liver did contain the apo-B,E(LDL) receptor with properties similar to the LDL receptor of extrahepatic cells. The observation of LDL binding activity was unexpected because the adult liver membranes used for the purification did not contain detectable

A.

B.
levels of LDL binding activity. This suggests that adult dog liver membranes possess the apo-B,E(LDL) receptor in quantities too low to detect by the membrane assay technique or that detergent solubilization unmasked the apo-B,E(LDL) receptor that was not reactive in the membrane binding assay.

The detergent solubilization and DEAE-cellulose purification also increased the amount of detectable apo-E HDL\textsubscript{c} binding activity compared to that in the 100,000 x g membrane fraction. The binding of \textsuperscript{125}I-apo-E HDL\textsubscript{c} displayed the same characteristics as the binding to the membrane fraction. The binding was high affinity (K\textsubscript{d} = 0.3–1.0 nM) and Ca\textsuperscript{2+}-dependent. The apo-E HDL\textsubscript{c} binding activity was sensitive to Pronase digestion (30 \mu g/ml) of proteins in the DEAE-purified fraction. Modifications of the arginine residues by cyclohexanedione or lysine residues by reductive methylation of the apo-E HDL\textsubscript{c} abolished the binding of the lipoproteins to receptors in the DEAE-purified fraction (data not shown). These results, along with the LDL binding data, demonstrated that the DEAE-purified fraction contained receptors that bind to both LDL and apo-E HDL\textsubscript{c}.

The receptor activity in the DEAE-purified fraction from liver membranes of cholesterol-fed dogs was compared to that obtained from liver membranes of normal adult dogs. Results indicated that much lower receptor activity for binding both LDL and apo-E HDL\textsubscript{c} was detected in the DEAE fraction isolated from the cholesterol-fed dogs (Fig. 3, A and B). The decrease in apo-E HDL\textsubscript{c} binding activity was probably due to the decrease in apo-B,E(LDL) receptors that is known to occur in liver membranes obtained from cholesterol-fed dogs (10, 25). It is important to note that the DEAE-purified fraction bound significantly more apo-E HDL\textsubscript{c} than LDL, suggesting that the apo-E HDL\textsubscript{c} binding activity was mediated by both the apo-B,E(LDL) receptor and the apo-E receptor in this DEAE fraction. Previously, it has been shown that the apo-B,E(LDL) receptor displayed three to four times more LDL binding activity than apo-E HDL\textsubscript{c} binding activity (57, 58). As will be shown later, the isolated hepatic apo-B,E(LDL) receptor likewise bound LDL and apo-E HDL\textsubscript{c} at a ratio of 3–4:1, whereas the isolated apo-E receptor lacked the ability to bind LDL. It was determined, using the 3–4:1 ratio of binding activity of LDL to apo-E HDL\textsubscript{c}, that the apo-B,E(LDL) receptor in the DEAE fraction accounted for ~200 ng of apo-E HDL\textsubscript{c} bound per mg of protein in the livers of normal, chow-fed adult dogs as compared to ~10 ng of apo-E HDL\textsubscript{c} bound per mg of protein in the livers of cholesterol-fed dogs. Therefore, only 20–25% of the apo-E HDL\textsubscript{c} binding activity in the normal liver DEAE fraction and ~10% of the apo-E HDL\textsubscript{c} binding activity in the cholesterol-fed liver DEAE fraction were attributed to binding to the apo-B,E(LDL) receptor. Thus, the majority of apo-E HDL\textsubscript{c} binding activity (75–80% in normal liver and ~90% in cholesterol-fed liver) appears to be mediated by the apo-E receptor in these fractions. Because of the high ratio of apo-E receptor to apo-B,E(LDL) receptor in liver from cholesterol-fed dogs, subsequent purification was carried out with liver membranes obtained from cholesterol-fed dogs.

The initial step in the purification of the hepatic apo-E receptor was to remove the apo-B,E(LDL) receptor using an LDL-Sepharose affinity column. This procedure resulted in the separation of the apo-B,E(LDL) receptor from the apo-E receptor. The hepatic apo-B,E(LDL) receptor, which bound to the LDL-Sepharose column, could be eluted with sodium succinate. A single peak with binding activities for both LDL and apo-E HDL\textsubscript{c}, was obtained (Fig. 4). The bound fraction demonstrated high affinity binding for both canine LDL and apo-E HDL\textsubscript{c}, as determined by Scatchard analysis of direct binding data (Table I). The apparent dissociation constants for both LDL and apo-E HDL\textsubscript{c} binding activity were similar to the values observed for the membrane-bound receptor detected by in vitro binding assays using fibroblast membranes and liver membranes of cholestyramine-treated dogs (10). The ratio of LDL to apo-E HDL\textsubscript{c} binding activity (3–4:1) indicated that the hepatic apo-B,E(LDL) receptor displayed the potential to bind multiple LDL particles, a property previously described for the extrahepatic apo-B,E(LDL) receptors (58).

The apo-E receptor was present in the fraction that did not bind to the LDL-Sepharose column. This unbound fraction displayed receptor binding activity for apo-E HDL\textsubscript{c}, but not

**TABLE I**

<table>
<thead>
<tr>
<th>Lipoprotein bound</th>
<th>Bound fraction (apo-B,E(LDL) receptor)</th>
<th>Unbound fraction (apo-E receptor)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_d ) ( pM )</td>
<td>( B_{max} ) ng/mg</td>
</tr>
<tr>
<td>\textsuperscript{125}I-LDL</td>
<td>28.0</td>
<td>24.2</td>
</tr>
<tr>
<td>\textsuperscript{125}I-Apo-E HDL\textsubscript{c}</td>
<td>0.4</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* NA, not applicable.
for LDL (Fig. 5). The characteristics of apo-E HDL<sub>c</sub> binding activity in the LDL-Sepharose-unbound fraction are also summarized in Table I. As shown, the apo-E receptor in the LDL-Sepharose-unbound fraction interacted with apo-E HDL<sub>c</sub> with high affinity binding activity. Furthermore, the binding was Ca<sup>2+</sup>-dependent and was sensitive to Pronase digestion. Interestingly, the apo-E HDL<sub>c</sub> binding activity in the LDL-Sepharose-unbound fraction could be inhibited by an antibody prepared against the apo-B,E(LDL) receptor. As shown in Fig. 6, the IgG inhibited apo-E HDL<sub>c</sub> binding activity, whereas the preimmune IgG had no effect on the binding activity of the LDL-Sepharose-unbound fraction. These results demonstrate that there are structural similarities, presumably at the apo-E-binding site, between the apo-B,E(LDL) and apo-E receptors. Therefore, the apo-B,E(LDL) receptor antibody can be used to identify and characterize both receptors.

Tentative identification of the canine apo-B,E(LDL) and apo-E receptors was accomplished by the immunoblotting technique using antibodies prepared against the bovine adrenal apo-B,E(LDL) receptor. The DEAE-cellulose fraction displayed three bands that were immunoreactive with the anti-apo-B,E(LDL) receptor IgG (Fig. 7). The 120,000-Da protein appeared to be a degradation product of the 130,000-Da apo-B,E(LDL) receptor. This conclusion was based on results obtained from the purification of both proteins on LDL-Sepharose affinity columns (Fig. 7). These data indicate that the canine hepatic apo-B,E(LDL) receptor is very similar to the extrahepatic apo-B,E(LDL) receptor, displaying an apparent <i>M</i><sub>a</sub> = 130,000 on nonreducing SDS-polyacrylamide gels. The third band was an additional immunoreactive protein in dog liver membranes that reacted with the anti-apo-B,E(LDL) receptor IgG. This protein, with an apparent <i>M</i><sub>a</sub> = 56,000, did not bind to the LDL-Sepharose column and was therefore identified as the apo-E receptor.

The identification of the 56,000-Da protein as the apo-E receptor was confirmed by its purification using an HDL<sub>c</sub>-Sepharose column. Most of the proteins in the LDL-Sepharose-unbound fraction did not bind to the HDL<sub>c</sub>-Sepharose column. The small amount of proteins that were bound to the HDL<sub>c</sub>-Sepharose column could be eluted with 20 mM sodium suramin (Fig. 8) or with 0.5 M NH<sub>4</sub>OH. The fraction eluted from HDL<sub>c</sub>-Sepharose affinity column possessed 125<sup>1</sup>-apo-E HDL<sub>c</sub> binding activity (Fig. 8) and lacked 125<sup>1</sup>-LDL binding activity. The unbound fraction lacked apo-E HDL<sub>c</sub> binding activity. The polysulfated hydrocarbon, sodium suramin, or ammonium hydroxide have been used effectively by Schneider et al. (28, 48) to displace LDL from the apo-B,E(LDL) receptor. The two reagents were also effective in the dissociation of apo-E HDL<sub>c</sub> from the apo-E receptor (Table II).

Proteins that were bound to the HDL<sub>c</sub>-Sepharose affinity column were analyzed on SDS-polyacrylamide gels. As shown by silver staining of the gels (Fig. 9), the HDL<sub>c</sub>-Sepharose-bound fraction contained a purified protein with a <i>M</i><sub>a</sub> = 56,000. Occasionally, a minor protein band with a <i>M</i><sub>a</sub> ~57,000 was also observed. This contaminant was identified as apo-E

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**Fig. 5.** Binding of 125<sup>1</sup>-LDL (○) and 125<sup>1</sup>-apo-E HDL<sub>c</sub> (△) to the apo-E receptor in the LDL-Sepharose-unbound fraction. An aliquot of the LDL-Sepharose-unbound fraction was precipitated by phosphatidylcholine and acetone and was used for the binding assay with 125<sup>1</sup>-LDL and 125<sup>1</sup>-apo-E HDL<sub>c</sub>. Specific binding of lipoproteins to the apo-E receptor was determined as described under "Experimental Procedures."

**Fig. 6.** Effect of bovine adrenal anti-apo-B,E(LDL) receptor IgG on 125<sup>1</sup>-apo-E HDL<sub>c</sub> binding to the apo-E receptor in the LDL-Sepharose-unbound fraction. Seventy micrograms of protein from the LDL-Sepharose-unbound fraction were precipitated with phosphatidylcholine and acetone and were used for the binding study with 125<sup>1</sup>-apo-E HDL<sub>c</sub>. The protein-phospholipid complex was incubated with anti-apo-B,E(LDL) receptor IgG (△) or with preimmune rabbit IgG (○) for 1 h at 23 °C before the addition of 2 μL of 125<sup>1</sup>-apo-E HDL<sub>c</sub> protein/mL. Specific binding of apo-E HDL<sub>c</sub> was determined as described under "Experimental Procedures."

**Fig. 7.** Immunoblots of apo-B,E(LDL) and apo-E receptors of canine liver membranes. Three hundred micrograms of protein from DEAE-purified fractions (lane 1) or LDL-Sepharose-bound (lane 2) and unbound fractions (lane 3) were applied to 7.5% SDS-polyacrylamide gels. The proteins were resuspended in buffer containing 62.5 mM Tris-HCl (pH 6.8), 3% SDS, and 10% glycerol (without reducing agent) prior to application to the gel. The proteins were transferred to nitrocellulose paper and were incubated with 50 μg/ml anti-apo-B,E(LDL) receptor IgG. Immunoreactive proteins were identified using an anti-rabbit 125<sup>1</sup>-IgG.
The presence of apo-E in this HDL,-Sepharose fraction was detected by immunoaffinity chromatography. The 56,000-Da apo-E receptor could be purified from the LDL-Sepharose-unbound fraction with high affinity to apo-E HDL, as described under "Experimental Procedures." The receptor was preincubated with 125I-apo-E HDL, as described under "Experimental Procedures." Each incubation tube contained 1 pg/ml either 125I-apo-E HDL, or cyclohexane-dione-modified 125I-apo-E HDL, or 50 pg/ml canine 125I-LDL. Where indicated, the anti-apo-E IgG (100 pg/ml) was added to the receptor-phospholipid complex 1 h prior to the addition of 125I-apo-E HDL, to form a receptor-phospholipid complex. The receptor was incubated with the radiolabeled lipoproteins with or without the indicated reagents for 1 h at 23 °C. The specific amounts of lipoproteins that bound to the receptor were determined as described under "Experimental Procedures." For the quantitation of binding activities, 125I-labeled apo-E receptors were used to determine the amount of apo-E receptor trapped in the filter.

The purified apo-E receptor was obtained by IgG-Sepharose chromatography of the HDL,-Sepharose-bound fraction as described in the legend to Fig. 9. The purified receptor was precipitated with phosphatidylincholine and acetone and used immediately for the binding assay as described under "Experimental Procedures." Each incubation tube contained 1 μg/ml either 125I-apo-E HDL, or cyclohexane-dione-modified 125I-apo-E HDL, or 5 μg/ml canine 125I-LDL. Where indicated, the anti-apo-E IgG (100 pg/ml) was added to the receptor-phospholipid complex 1 h prior to the addition of 125I-apo-E HDL, to form a receptor-phospholipid complex. The receptor was incubated with the radiolabeled lipoproteins with or without the indicated reagents for 1 h at 23 °C. The specific amounts of lipoproteins that bound to the receptor were determined as described under "Experimental Procedures." For the quantitation of binding activities, 125I-labeled apo-E receptors were used to determine the amount of apo-E receptor trapped in the filter.

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apo-E-containing lipoproteins. Both chylomicron remnants (β-VLDL fraction I) and VLDL remnants (β-VLDL fraction II) were capable of inhibiting 125I-apo-E HDL₄ binding to the purified apo-E receptor (Fig. 10). The specificity of the apo-E receptor was further confirmed by the ability of an apo-E monoclonal antibody (1D7) to inhibit the binding of 125I-apo-E HDL₄ to the purified apo-E receptor. This anti-apo-E IgG has previously been shown to block apo-E binding to lipoprotein receptors (21, 22) (Table I). In addition, cyclohexane-purified apo-E receptor (Fig. 10). The specificity of the apo-E HDL₄ binding activity was observed (Table I). Immunoprecipitation of the apo-E receptor. As shown in Table IV, a large fraction of the apo-E HDL₄ binding activity was removed by immunoprecipitation. The residual apo-E HDL₄ binding activity in the DEAE fraction after immunoprecipitation was due to the apo-B,E(LDL) receptor in this fraction. This conclusion is supported by the following observations: immunoprecipitation using the anti-apo-E receptor IgG had no effect on LDL binding activity in the DEAE fraction; and the antibody against the 56,000-Da protein abolished binding to the receptor (previously this procedure has been shown to prevent HDL₄ binding to apo-B,E(LDL) and apo-E receptors (10)). Furthermore, high concentrations of canine LDL were incapable of displacing the 125I-apo-E HDL₄ (2 μg of protein/ml) or 125I-LDL (20 μg of protein/ml) from HDL₄ to the purified apo-E receptor. This anti-apo-E IgG has previously been shown to prevent HDL₄ binding to apo-B,E(LDL) and apo-E receptors (10)). Furthermore, high concentrations of canine LDL were incapable of displacing the 125I-apo-E HDL₄ (2 μg of protein/ml) or 125I-LDL (20 μg of protein/ml) from HDL₄ to the purified apo-E receptor. Therefore, these re-

![Fig. 10. Competitive displacement of 125I-apo-E HDL₄ from binding to the purified canine apo-E receptor. Ten micrograms of apo-E receptor were incubated with 125I-apo-E HDL₄ (0.3 μg of protein/ml) in the presence or absence of unlabeled apo-E HDL₄ (□), chylomicron remnants (○), or VLDL remnants (△). Chylomicron and VLDL remnants were isolated from the plasma of cholesterol-fed dogs. The amount of specific 125I-apo-E HDL₄ bound to the purified apo-E receptor was determined as described under "Experimental Procedures."](image)

**Table IV**

<table>
<thead>
<tr>
<th>Immunoprecipitation of apo-E receptor</th>
<th>Apo-E HDL₄ bound</th>
<th>LDL bound</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DEAE fraction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>191.6 (100)</td>
<td>32.1 (100)</td>
</tr>
<tr>
<td>+ Preimmune IgG</td>
<td>165.2 (87)</td>
<td>28.9 (90)</td>
</tr>
<tr>
<td>+ Anti-apo-E receptor IgG</td>
<td>7.8 (4)</td>
<td>30.5 (96)</td>
</tr>
<tr>
<td><strong>LDL-Sepharose-unbound fraction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>166.8 (100)</td>
<td>0</td>
</tr>
<tr>
<td>+ Preimmune IgG</td>
<td>226.3 (136)</td>
<td>0</td>
</tr>
<tr>
<td>+ Anti-apo-E receptor IgG</td>
<td>2.7 (2)</td>
<td>0</td>
</tr>
</tbody>
</table>

![Fig. 11. Immunoblot of purified apo-B,E(LDL) receptor (A) and purified apo-E receptor (B) with anti-apo-E receptor IgG. The apo-B,E(LDL) receptor was purified on an LDL-Sepharose column, and the apo-E receptor was purified by HDL₄-Sepharose column chromatography. The receptors were applied to 7.5% SDS-polyacrylamide gels in the absence of a reducing agent. Proteins were transferred to nitrocellulose paper and were incubated with 50 μg/ml anti-apo-E receptor IgG.](image)
apo-B,E(LDL) receptor (A) or apo-E receptor coated on the microtiter wells. IgG was used to determine the amount of specific IgG bound to the receptors coated on the microtiter wells.

**FIG. 12.** Solid phase radioimmunoassay of purified apo-B,E(LDL) receptors and purified apo-E receptors. The purified apo-B,E(LDL) receptor (A) or apo-E receptor (B) was coated on microtiter wells and incubated with either anti-apo-B,E(LDL) receptor IgG or anti-apo-E receptor IgG. A $^{125}$I-labeled anti-rabbit IgG was used to determine the amount of specific IgG bound to the receptors.

The observation that the apo-E receptor displayed similar electrophoretic mobility in the presence or absence of dithiothreitol suggested that the apo-E receptor may also differ from the apo-B,E(LDL) receptor in cysteine content. Analysis of the amino acid composition of the purified apo-E receptor confirmed this possibility. As shown in Table V, the canine apo-E receptor contained only 1.1% cysteine in contrast to a 7.3% cysteine content observed for the human apo-B,E(LDL) receptor (31). Therefore, although the apo-E receptor possesses certain characteristics resembling the apo-B,E(LDL) receptor, the apo-E receptor is a unique protein that is structurally and functionally distinct from the apo-B,E(LDL) receptor.

In addition to the purification and characterization of the canine hepatic apo-E receptor, the apo-E receptor was also isolated from human liver membranes for comparison. The same protocol as described above was also used to purify a single protein with a $M_r = 56,000$ from human liver membranes (Fig. 14). The purified human apo-E receptor cross-reacted with antibodies prepared against the canine apo-E receptor (Fig. 14). In addition, the human apo-E receptor also reacted with the anti-apo-B,E(LDL) receptor IgG (Fig. 14). Amino acid analysis of the purified human apo-E receptor revealed similarities with the canine apo-E receptor and revealed significant differences in amino acid composition as compared with the human apo-B,E(LDL) receptor (Table V). In addition to the difference in cysteine content between the human apo-B,E(LDL) and apo-E receptors, significant differences were also observed for the amino acids Ser, Glu, Ala, and Met (Table V).

**FIG. 13.** Effect of reducing agent on the electrophoretic mobility of the purified canine apo-B,E(LDL) receptor (A) and the purified apo-E receptor (B). The purified apo-B,E(LDL) receptor and the apo-E receptor were incubated with buffer containing 625 mM Tris-HCl (pH 6.8), 3% glycerol, and 3% SDS in the absence (−DTT) or presence (+DTT) of 100 mM dithiothreitol (DTT) for 15 min at 23 °C. The samples were then analyzed on 7.5% SDS-polyacrylamide gels as described under “Experimental Procedures.”

Hepatic Apo-E (Remnant) Receptor

The observation that the apo-E receptor displayed similar electrophoretic mobility in the presence or absence of dithiothreitol suggested that the apo-E receptor may also differ from the apo-B,E(LDL) receptor in cysteine content. Analysis of the amino acid composition of the purified apo-E receptor confirmed this possibility. As shown in Table V, the canine apo-E receptor contained only 1.1% cysteine in contrast to a 7.3% cysteine content observed for the human apo-B,E(LDL) receptor (31). Therefore, although the apo-E receptor possesses certain characteristics resembling the apo-B,E(LDL) receptor, the apo-E receptor is a unique protein that is structurally and functionally distinct from the apo-B,E(LDL) receptor.

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Immunoblotting with the anti-apo-E receptor IgG and the anti-apo-B,E(LDL) receptor was used to determine the presence or absence of the apo-E receptor and the apo-B,E(LDL) receptor in different cell types. As summarized in Table VI, both the apo-B,E(LDL) and the apo-E receptors were present.

**TABLE V**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Canine apo-E receptor</th>
<th>Human apo-E receptor</th>
<th>Human apo-B,E(LDL) receptor $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol %</td>
<td>mol %</td>
<td>mol %</td>
</tr>
<tr>
<td>Cys</td>
<td>1.1</td>
<td>1.0</td>
<td>7.3</td>
</tr>
<tr>
<td>Asp</td>
<td>8.8</td>
<td>8.8</td>
<td>8.7</td>
</tr>
<tr>
<td>Thr</td>
<td>5.3</td>
<td>5.4</td>
<td>5.8</td>
</tr>
<tr>
<td>Ser</td>
<td>5.3</td>
<td>5.9</td>
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<tr>
<td>Glu</td>
<td>11.4</td>
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<tr>
<td>Pro</td>
<td>4.7</td>
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<td>4.5</td>
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<tr>
<td>Gly</td>
<td>11.9</td>
<td>9.4</td>
<td>7.2</td>
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<tr>
<td>Ala</td>
<td>8.9</td>
<td>8.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Val</td>
<td>7.2</td>
<td>6.6</td>
<td>6.7</td>
</tr>
<tr>
<td>Met</td>
<td>1.8</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Ile</td>
<td>5.7</td>
<td>5.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Leu</td>
<td>8.7</td>
<td>9.0</td>
<td>7.7</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.7</td>
<td>3.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Phe</td>
<td>3.3</td>
<td>3.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Lys</td>
<td>6.4</td>
<td>7.5</td>
<td>7.7</td>
</tr>
<tr>
<td>His</td>
<td>1.7</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Trp</td>
<td>ND$^b$</td>
<td>ND</td>
<td>2.3</td>
</tr>
<tr>
<td>Arg</td>
<td>5.4</td>
<td>5.6</td>
<td>5.3</td>
</tr>
</tbody>
</table>

$^a$ Derived from Ref. 31.

$^b$ ND, not determined.
The identification of human apo-E receptor. The identical procedure used for purification of canine apo-E receptor was used to purify apo-E receptor from human liver membranes. The human apo-E receptor obtained after purification on HDL-Sepharose and immunoaffinity chromatography on an anti-apo-E receptor IgG-Sepharose column was applied to 10% SDS-polyacrylamide gels. Lane 1 shows the silver-stain picture of the purified human apo-E receptor; lane 2 shows the human apo-E receptor blotted with anti-apo-E receptor IgG; and lane 3 shows the receptor reactivity with anti-apo-B,E(LDL) receptor IgG.

Table VI
Cell distribution of the apo-E receptor

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Apo-B,E(LDL) receptor</th>
<th>Apo-E receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Humans</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rats</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mice</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Detergent-solubilized cell extracts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat liver parenchymal cells</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table VI shows the receptor reactivity with anti-apo-B,E(LDL) receptor IgG. Two hundred micrograms of protein from SDS-solubilized liver membranes (100,000 x g pellet) or from cell extracts from fibroblasts and liver parenchymal cells were applied to 10% SDS-polyacrylamide gels and immunoblotted with 20 μg/ml anti-apo-B,E(LDL) receptor or anti-apo-E receptor to identify the apo-B,E(LDL) receptor (M<sub>r</sub>, ~130,000) and the apo-E receptor (M<sub>r</sub>, ~56,000).

not bind significant quantities of LDL (10, 25); 2) the binding of apo-E HDL<sub>α</sub> to the liver membranes was not inhibited by excess unlabelled LDL (10); and 3) the expression of the apo-B,E(LDL) receptor on liver membranes was subject to regulation by metabolic manipulation, whereas apo-E receptor expression was independent of metabolic control (25, 27).

In the present study, we report the isolation and purification of both apo-B,E(LDL) and apo-E receptors from canine and human liver membranes. The hepatic apo-B,E(LDL) receptor is similar, or identical, to the extrahepatic apo-E receptor and displays an apparent M<sub>r</sub> = 130,000 on nonreducing SDS-polyacrylamide gels (33). The apo-E receptor is a distinct protein with an apparent M<sub>r</sub> = 56,000.

The initial hurdle in the purification procedure was the solubilization of functional receptors from the liver membranes. The most effective reagent was the zwitterionic detergent CHAPS (56). This detergent is nondenaturing and is very effective in the prevention of protein-protein aggregation (56). Other advantages of using CHAPS include its compatibility with ion exchange separation techniques and its high critical micellar concentration (56). The latter property made it possible to precipitate the receptor using the phospholipid/acetone method (28) and to test the binding of lipoproteins to the receptor. Other detergents tested, such as octyl-β-glucoside, dodecyl maltoside, Nonidet P-40, and Tween 20, failed to extract the apo-E receptor from the liver membranes. Membranes solubilized with Triton X-100, Triton N-101, or a mixture of octyl glucoside and dodecyl maltoside did demonstrate apo-E HDL<sub>α</sub> binding activity. However, the solubilized proteins formed microaggregates in solution and thereby prevented further purification of the apo-E receptor.

Detergent-solubilized liver membranes displayed binding activity for both LDL and apo-E HDL<sub>α</sub>. As discussed above, the solubilization step probably unmasked the low number of apo-B,E(LDL) receptors in the liver membranes of adult or cholesterol-fed dogs. Previously, Schneider et al. (28) have shown that detergent solubilization of the adrenal membranes also results in the unmasking of LDL receptors previously undetected by the membrane assay technique. The present study demonstrated that the hepatic apo-B,E(LDL) receptor, purified by affinity chromatography on an LDL-Sepharose column, displays characteristics identical to the apo-B,E(LDL) receptors of extrahepatic cells: 1) high affinity binding of both apo-B-containing LDL and apo-E HDL<sub>α</sub>; 2) an ~20-fold greater affinity for the apo-E HDL<sub>α</sub> as compared to LDL; 3) a ratio of binding of LDL/HDL<sub>α</sub> of 3–4:1, which has been interpreted as demonstrating the existence of multiple binding sites on the apo-B,E(LDL) receptor molecule (58); and 4) an apparent M<sub>r</sub> = 130,000 on nonreduced gels, which is similar to that of the extrahepatic apo-B,E(LDL) receptor.

After removal of the apo-B,E(LDL) receptor using an LDL-Sepharose affinity column, the hepatic apo-E receptor was purified from the LDL-Sepharose-unbound fraction by a two-step procedure using HDL<sub>α</sub>-Sepharose and anti-apo-E receptor IgG-Sepharose affinity columns. The purified protein displayed a M<sub>r</sub> = 56,000 on SDS-polyacrylamide gels and had characteristics identical to the apo-E receptor on liver membranes. The 56,000-Da apo-E receptor bound to apo-E HDL<sub>α</sub> specifically and did not interact with normal canine LDL. Furthermore, the binding of apo-E HDL<sub>α</sub> to the purified apo-E receptor was inhibited by antibodies against apo-E or by modification of arginine residues of apo-E, indicating the importance of this apolipoprotein in mediating binding to the purified apo-E receptor.

The immunologic similarities between the apo-B,E(LDL)
receptor and the apo-E receptor, as demonstrated by the reactivity of anti-apo-B,E(LDL) receptor IgG with both proteins, are not surprising observations. The ability of the apo-B,E(LDL) and apo-E receptors to interact with apo-E clearly indicates the similarities between these lipoprotein receptors. In addition, the variant forms of apo-E that display an abnormal ability to interact with apo-B,E(LDL) receptors likewise are defective in binding to the apo-E receptor (22, 23), indicating that the two receptors interact with the E apolipoprotein at the same site within the apolipoprotein molecule. The ability of a monoclonal antibody to apo-E to inhibit apo-E HDL$_r$ binding to both receptors further suggests that the apo-B,E(LDL) and apo-E receptors both bind to the E apolipoprotein through the same binding domain (22). Taken together, these data indicate that the lipoprotein-binding site(s) on the apo-E receptor are very similar to those of the apo-B,E(LDL) receptor. The ability of the anti-apo-B,E(LDL) receptor IgG to inhibit apo-E HDL$_r$ binding to the apo-E receptor further establishes the similarities of at least certain domains within the apo-B,E(LDL) and apo-E receptors.

Although the apo-E receptor appears to share many similar characteristics with the apo-B,E(LDL) receptor, it is important to note that the two receptors are structurally and functionally distinct proteins. The ability of the apo-B,E(LDL) receptor, but not the apo-E receptor, to interact with the apo-B of LDL is an important functional distinction. Major differences are also apparent in the structure of the apo-E receptor as compared to the apo-B,E(LDL) receptor. Specific antibodies raised against the apo-E receptor did not react with the apo-B,E(LDL) receptor. This observation suggests the presence of distinct antigenic epitope(s) in the apo-E receptor that are not present in the apo-B,E(LDL) receptor. The lack of immunoreactivity of the specific apo-E receptor antibody with the apo-B,E(LDL) receptor was observed in assays with the native (Fig. 12) and denatured (Fig. 11) receptor, thus establishing that the lack of reactivity was not due simply to a conformational difference between the two proteins. Furthermore, a comparison of the amino acid analyses of the human apo-E receptor and the apo-B,E(LDL) receptor supports the uniqueness of these two receptors. Therefore, it is likely that a major portion of the primary structure of the apo-E receptor is different from that of the apo-B,E(LDL) receptor.

The purification of the apo-E receptor and the identification of specific antibodies against the apo-E receptor will facilitate the determination of its in vivo function. However, in view of the ability of this hepatic apo-E receptor to bind chylomicron remnants, the critical role of apo-E in the hepatic uptake of chylomicron remnants, and the localization of the apo-E receptor to the liver parenchymal cell, it is reasonable to postulate that the apo-E receptor is the chylomicron remnant receptor responsible for the in vivo clearance of remnant lipoproteins.

Acknowledgments—We greatly appreciate the excellent technical assistance of Dennis Miranda, Peter Lindquist, and Jim Leffler. The amino acid analysis was performed by C. Roy C. Rall Jr. Rat hepatocyte cultures were provided by Dr. D. Montgomery Bissell and his associates at the Rice Liver Center, University of California at San Francisco. We also thank Barbara Allen and Sally Gullatt Seehafer for editorial assistance, James X. Warter and Norma Jean Gargaus for graphics, and Sylvia Richmond and Kate Shelly for manuscript preparation.

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Hepatic Apo-E (Remnant) Receptor