Distribution and characterization of the serum lipoproteins and apoproteins in the mouse, *Mus musculus*

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Abstract Murine lipoproteins were separated into nine subfractions by a density gradient ultracentrifugal procedure. They were characterized by electrophoretic, immunological, chemical, and morphological analyses, and their protein moieties were defined according to charge, molecular weight, and isoelectric point. HDL predominated (~500 mg/dl serum), the mode of its distribution being situated in the d 1.09–1.10 g/ml (F 1.21 ~ 4) region. Chemical analysis showed subfractions of d 1.085–1.136 g/ml to resemble human HDL_3 closely, including the presence of apoA-I (M_r 25,000–27,000) as their major apolipoprotein. An apoA-II-like protein, of M_r 8400 (in monomeric form), was also tentatively identified. In electrophoretic mobility and chemical composition, the d 1.060–1.085 g/ml subfraction (~10% of total HDL) was distinct and akin to human HDL_2. ApoA-I represented ~60% of its complement of low molecular weight apoproteins. The density range used for separation of human HDL_2 (d 1.066–1.100 g/ml) by gradient ultracentrifugation is inadequate in the mouse, and the d 1.060–1.085 g/ml interval is more appropriate. The 1.063 g/ml boundary for separation of mouse LDL from HDL was unsuitable. Immunological and electrophoretic studies revealed that α-migrating lipoproteins were present in the d 1.046–1.060 g/ml range, a finding consistent with their enrichment in apoA-I; apoE-, apoA-II-, and apoC-like proteins were also detected. These findings indicate the presence of HDL_1 particles. Murine apoA-I and apoB-like proteins of higher ($apoB_H$) and lower ($apoB_L$) molecular weight were constituents of the d 1.033–1.046 g/ml fraction. Alternative techniques, such as electrophoresis in starch block, are therefore a prequisite for separation of apoB from α-migrating, apoA-I-containing lipoproteins in the low density range in mouse serum. The LDL class (d 1.023–1.060 g/ml) amounted to only ~20% of the total murine lipoproteins of d < 1.188 g/ml (65–70 mg/dl serum). Particles were richer in triglyceride, larger in diameter (mean 244 Å), and more heterogeneous than typical of man. VLDL (40–80 mg/dl serum) was triglyceride-rich (66% by weight) and similarly heterogeneous in size (mean diameter 494 Å; range 270–750 Å). $ApoB_H$ and $apoB_L$ were prominent in murine VLDL, and cross-reacted with an antiserum to human apoB. ApoE- and apoA-I-like proteins were also detectable in apoVLDL, as was a protein of 70,000–75,000 mol wt. The presence of murine apolipoproteins analogous to human apoB and apoE was confirmed by the immunological cross-reactivities of VLDL and LDL with monospecific antisera to the human proteins. The marked similarity of lipoprotein and apolipoprotein profile in the mouse and rat is notable. Since murine VLDL contains apoE and $apoB_L$, this resemblance may extend to the metabolism of chylomicron remnants and hepatic VLDL in the two species.—**Camus, M-C., M. J. Chapman, P. Forgez, and P. M. Laplaud.** Distribution and characterization of the serum lipoproteins and apoproteins in the mouse, *Mus musculus*. *J. Lipid Res.* 1983. **24:** 1210–1228.

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As an animal model in which to study the genetic basis of metabolic disease, the mouse, *Mus musculus,* is perhaps unequalled, since amongst the mammals, no other species presents such a vast array of genetically-defined inbred strains. Somewhat surprisingly, inherited disorders of lipid metabolism in the mouse have received little attention. Indeed, our knowledge of the lipid transport system in healthy normolipidemic mice is cursory in comparison to that of other rodents, such as the rat, *Rattus norvegicus,* and guinea pig, *Cavia porcellus* (1).

Abbreviations: VLDL, very low density lipoproteins, of density as defined; IDL, intermediate density lipoproteins, of density as defined; LDL, low density lipoproteins, of density as defined; HDL, high density lipoproteins and HDL subfractions, HDL_1, HDL_2, HDL_3, of density as defined; VHDL, very high density lipoproteins; PMSF, phenylmethylsulfonyl fluoride; apo, apolipoprotein; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; TMU, tetramethylurea, S_f, flotation coefficient in an NaCl medium of density 1.063 g/ml; F, flotation coefficient in an NaCl–NaBr medium of density 1.21 g/ml.

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Most of the information available on the lipid transport system of *Mus musculus* is qualitative in nature, consisting primarily of electrophoretic analyses (2–5). To date, separation of lipoproteins from murine plasma has been achieved by sequential ultracentrifugation (6), followed by agarose gel chromatography (7, 8), polyacrylamide gel electrophoresis (9), and by polyanion precipitation (9). More recently, rate zonal ultracentrifugation has been used to define the lipoprotein profiles in two inbred strains (C57 BR/cdJ and CBA/J), which differ in their susceptibility to diet-induced atherosclerosis (10).

To our knowledge, only the studies of Mathur and Spector (8) and of Morrisett et al. (10) have provided data on murine apolipoproteins. In the former study (8), observations were made on animals bearing ascites tumours and the relationship of the apolipoproteins in the ascites fluid to those of normal, healthy mice is therefore indeterminate. In the latter study (10), a polypeptide analogous to human apoA-I was identified as the major protein component of murine HDL on the basis of its electrophoretic mobility in SDS-polyacrylamide gel, while the presence of an apolipoprotein resembling human apoE was suggested by detection of a serum component that reacted immunologically with antiserum to the rat protein.

As a prelude to investigating the effect of nutritional factors on the lipid transport system of certain inbred strains of mouse, we judged it essential to establish the lipoprotein and apolipoprotein profile typical of the genetically normal, normolipidemic strain—in our case, the Swiss. The purpose of the present study was therefore to determine the density distribution and qualitative and quantitative characteristics of the serum lipoproteins and apoproteins in the adult Swiss mouse (*Iffa Credo*), following their fractionation by a single-step, gradient density ultracentrifugal procedure (11).

MATERIALS AND METHODS

Materials

Animals and diets

Mature male mice of the Swiss OF1 strain (*Iffa Credo*), and aged 3 months, were used. Upon arrival in the laboratory, animals were caged in groups of five, and maintained at ambient temperature (22 ± 1°C) with a 12-hr light cycle. The mice had free access to water and food until the day before they were killed. They were fed a control diet containing 1364.8 Joules/100 g; its chief components (as percent weight) were 77.2% wheat flour, 3.0% bran, 12.1% cow milk casein, 0.4% DL-methionine, 1.1% lard, 4.0% salt mixture (12), and 2.2% of the vitamin mixture of Lemmonier et al. (13).

Blood samples

Blood from unanesthetized animals, fasted overnight for approximately 16 hr, was drawn by retroorbital venous plexus puncture with a narrow-bore Pasteur pipette and collected separately. After clotting at room temperature for 2 hr, sera were separated following low speed centrifugation at 4°C and randomly pooled. Antibacterial agents (0.001% sodium merthiolate and 0.01% sodium azide) were immediately added to the various samples of pooled sera (each corresponding to approximately ten sera, of an average of 0.4 ml/mouse). They were stored at 4°C, and isolation of lipoproteins was normally carried out within 72 hr. In certain instances, PMSF was added to the serum upon separation to a final concentration of 1 mM; in such cases, 1 mM PMSF was included in all density solutions used for lipoprotein isolation.

Methods

Isolation of lipoproteins

Serum lipoproteins were separated by both flotational and gradient density ultracentrifugation. These two procedures were used to define the density distribution of the major classes of mouse lipoproteins and to determine whether the classical conditions of sequential ultracentrifugation applicable to human lipoproteins (14) were valid for those of the mouse.

Analytical ultracentrifugation

This type of analysis was performed to quantitate low density ($d < 1.063$ g/ml) and high density lipoproteins (d 1.063–1.21 g/ml) as described by Laplaud, Beaubatie, Maurel (15), and Ewing et al. (16).

Gradient density ultracentrifugation

Lipoprotein fractions were isolated by a single spin–discontinuous gradient method as outlined by Chapman et al. (11).

The gradients were formed in cellulose nitrate tubes ($^9/_{16}$″ diam × 3 $^1/_2$″ length) after adjustment of the nonprotein solvent density of each sample of pooled sera to 1.21 g/ml with solid KBr (0.325 g/ml of serum). Gradient construction was carried out at ambient temperature with the use of a Buchler Autodensiflow II (Buchler Instruments, Searle Analytic, Inc., Fort Lee, NJ) coupled to a Gilson Minipuls II peristaltic pump at a speed of 1 ml/min. Each tube was immediately placed in the swinging bucket Beckman SW 41 rotor and spun in a Sorvall model OTD-50 ultracentrifuge in the Reograd/ARC-slow mode at 40,000 rpm (56.7×10^7 *g*-avg min) for 48 hr at 15°C, using no brake at the end of the run.

Eleven fractions of an average of 1 ml (with the exception of fraction 12, about 1.5 ml) were collected

from the meniscus of each tube downwards by aspiration with a narrow-bore Pasteur pipette. These fractions were then dialyzed in Spectrapor tubing (Spectrum Medical Industries, Los Angeles, CA; exclusion limit ca. 3,500) for 3 × 24 hr at 4°C against a solution containing 0.05 M NaCl, 5 mM Tris-HCl, 0.04% EDTA, 0.2% sodium azide, and 0.005% sodium merthiolate at pH 7.4.

Characterization of serum lipoprotein fractions

Lipoprotein fractions and whole serum corresponding to each gradient were characterized by electrophoretic, immunological, chemical, and morphological analyses. The protein moieties of lipoprotein fractions were defined according to the charge, molecular weight, and isoelectric point of their constituent apolipoproteins.

Electrophoresis

Samples (5 μl) of whole serum and lipoprotein fractions were prestained with Sudan black (v/v) and then electrophoresed on polyacrylamide gel slabs ("Lipofilm", Sebia, Issy-les-Moulineaux, France) for 1 hr at 250 V and 15 mA/slab. These slabs were constructed to give a discontinuous gradient from 2% (at the area of application) to 3% (in the running gel).

Immunological methods

Double immunodiffusion and immunoelectrophoresis were carried out by the techniques of Ouchterlony (17) and Scheidegger (18), respectively, in 1% agarose in 0.025 M veronal buffer (pH 8.6). Monospecific antisera to human apoA-I and to human apoE were prepared in rabbits; a rabbit antiserum to human apoB was kindly provided by Dr. S. Goldstein.

Duplicate samples of whole serum and of the various fractions were electrophoresed against an antiserum to mouse serum produced in the rabbit (no. 65014, Miles Laboratories, Paris, France) with water-cooling at 4 V/cm, for 60 min. After 72 hr at 4°C in wet chambers containing an antibacterial agent (0.01% sodium azide), the slides were washed with 0.9% NaCl for 2 days. After drying in air, each sample slide was stained for protein (amido Schwarz) and lipid (Sudan black).

Chemical analysis

Total and free cholesterol were determined according to the enzymatic method of Röschlau, Bernt, and Gruber (19), using Boehringer/Mannheim GmbH Kits no. 237574 and 310328, respectively. Cholesteryl esters were calculated by multiplying the amount of esterified cholesterol by 1.67; this latter was determined as the difference between total and free cholesterol. In this calculation, it was assumed that the factor of 1.67, corresponding to the ratio of the average molecular weight of human cholesteryl ester to that of free cholesterol, was the same in the mouse. This assumption is in fact largely consistent with the calculated factor for the fatty acids of murine plasma cholesteryl esters (i.e., 1.68) and based on the data of Rehnborg, Nichols, and Ashikawa (20).

Triglycerides were estimated by the enzymatic method of Eggstein (21), employing Boehringer/Mannheim GmbH Kit no. 124966, which determines the total glycerol content of a sample after its saponification with ethanolic KOH. Phospholipids were assayed directly with the "phospholipid B test Wako" (Biolyon Bp, 69570 Dardilly, France); this method measures the choline content of all choline-containing phospholipids. In the phospholipids of vertebrate lipoproteins, choline-containing components typically account for 90–95% of the total (1). Lipoprotein protein contents were determined by the method of Lowry et al. (22) using bovine serum albumin (Sigma) as standard. We have previously reported on the reproducibility and precision of these analyses (11).

Morphological analysis

Samples of lipoprotein fractions from the density gradient were examined by electron microscopy after negative staining with 2% potassium phosphotungstate at pH 7.6 (11). The diameters of up to 300 particles were subsequently measured on each of up to three negatives (or prints) and the frequency distribution of particle sizes was plotted.

Analysis of protein moieties

Electrophoresis according to charge at alkaline pH. The tetramethylurea-soluble apolipoproteins of ultracentrifugally separated lipoprotein fractions were electrophoresed according to the method of Kane (23). Approximately 50 μg or 100 μg of TMU-soluble protein of density-gradient fractions, 1, 4, 5, 6, 7, 8, 9, and 10, respectively, were applied to each polyacrylamide gel (7.5% acrylamide monomer containing 8 M urea at pH 8.9).

Samples were electrophoresed in a Buchler disc-gel system (Searle Analytic, Inc.) at 2.5 mA/tube with water cooling. Gels were fixed and subsequently stained with Coomassie Brilliant Blue R as described earlier (24). Densitometric scanning of stained gels was carried out at 550 nm on a Sebia Cello-system no. 134, which integrates the surface area of each peak and calculates the percentage of total staining due to each band. The limitations of this approach in quantifying apolipoproteins were discussed earlier (24).

In order to compare the migration of specific apolipoprotein bands in one electrophoretic system with that in another, the following procedure was adopted. Urea-soluble apolipoproteins (non-apoB) were first electrophoresed in a series of alkaline–urea gels (23) (see

above); electrophoresis was terminated when the dye front had obtained a fixed position at the bottom of each gel. One gel was rapidly stained with a solution of 0.04% (w/v) Coomassie Brilliant Blue G250 in 3.5% (w/v) perchloric acid (25). A slice was then cut from each of three or four unstained gels in a region corresponding to a given band in its stained counterpart. The gel slices were crushed and their apolipoprotein content was eluted electrophoretically essentially by the procedure of Stephens (26); the buffer system of Kane (23) was used in the latter elution, with the exception that the glycine content of the upper buffer was replaced by boric acid. The protein eluate was extensively dialyzed against a 5 mM solution of NH_4HCO_3 and lyophilized. Aliquots (ca. 100 μg) were electrophoresed in the SDS and isoelectric focusing systems described below.

Electrophoresis in SDS for molecular weight estimation. The molecular weight of the apolipoproteins was estimated by electrophoresis in SDS-polyacrylamide gel according to Weber and Osborn (27). Samples of the dialyzed lipoprotein fractions (containing 100 μg of protein) were either used directly in cases when the protein concentration was >100 μg/100 μl, or in the case of more dilute samples, were partially lyophilized to reduce the volume until a similar concentration was obtained. An equal volume of a solution containing SDS (3% w/v), 1% (w/v) β-mercaptoethanol, and 0.01 M sodium phosphate at pH 7.0 was then added to each, and the protein was delipidated and solubilized by heating at 90°C for 3 min. Glycerol (1 drop) and bromophenol blue were added prior to application of a volume equivalent to 50–100 μg of protein to the gel.

Monomer acrylamide concentrations were 4.4% and 10%; apoVLDL and apoLDL were analyzed at the former and apoHDL at the latter concentration.

For determinations of molecular weight (27), two calibration curves were constructed with polymerized molecular weight markers (BDH Biochemicals Ltd, Poole, U.K.) ranging from 14,300 to 71,500 for gels of 10% monomer and from 53,000 to 318,000 for those of 4.4%. Representative correlation coefficients for these curves were −0.9910 and −0.9959, respectively. Samples (~50 μg) of molecular weight standards were pretreated in the same way as those containing apolipoproteins in each procedure. On account of the small quantities of lipoprotein protein in certain density-gradient fractions, such as 4 and 5, or of the minimal available volumes of others, it was necessary to pool fractions 4 and 5 (as LDL) and fractions 7, 8, and 9 (as HDL_3).

Gels were stained and destained by the method of Karlson et al. (28).

Analytical isoelectric focusing for determination of pI values. Analytical isoelectric focusing was performed according to Pagnan et al. (29) in polyacrylamide gels (7.5% monomer) containing 6 M urea and 2% ampholine of pH 4–6.5 (Pharmacia Fine Chemicals). Samples (100–150 μg of protein) were applied to the gels (10.5 cm long, 0.6 cm diameter) in 100 μl of 6 M urea, 20 mM N-ethyl morpholine (SDS, Vitry-sur-Seine, France), and 20% (w/v) sucrose, onto which was layered the upper electrolyte solution (0.1 M glycine) at pH 7.0. The anodic, lower chamber contained 0.01 M Hepes at pH 3.50. Focusing was carried out at a constant voltage of 400 V for 5½ hr at 10°C. Gels were stained with Coomassie Brilliant Blue as outlined by Karlson et al. (28). The pI values of individual stained fractions were assessed by measurement of the pH of aqueous eluates of slices (0.5-cm thick) cut from unstained reference gels, from which a calibration curve was constructed.

RESULTS

Lipid and lipoprotein content of whole serum

The concentrations of lipids in pooled mouse sera are shown in **Table 1.** Elevated triglyceride levels were found in these animals, despite fasting for 16 hr. Electrophoresis of sera on polyacrylamide gel slabs (**Fig. 1**) revealed two bands upon staining with Sudan black. The

TABLE 1. Total concentrations of the major lipids of mouse serum

Total Cholesterol[a]	Cholesteryl Ester[b]	Free Cholesterol	Triglyceride[c]	Phospholipid
		mg/dl serum[d]		
95.0 ± 3.0	110.2 ± 3.6	28.7 ± 2.8	214.5 ± 1.7	253.0 ± 7.1

[a] Values for total cholesterol represent the sum of free and esterified cholesterol.
[b] Values of cholesteryl ester correspond to the difference between those of total cholesterol and free cholesterol multiplied by a coefficient of 1.67.
[c] Determined as total glycerol after saponification; ~6% (i.e., ~13 mg/dl) was accounted for by the free glycerol content of whole serum.
[d] Values are the mean ± SEM of duplicate analyses of four pools of mouse sera representing an average of ten animals per pool; the same pools were used for lipoprotein isolation by density gradient ultracentrifugation.

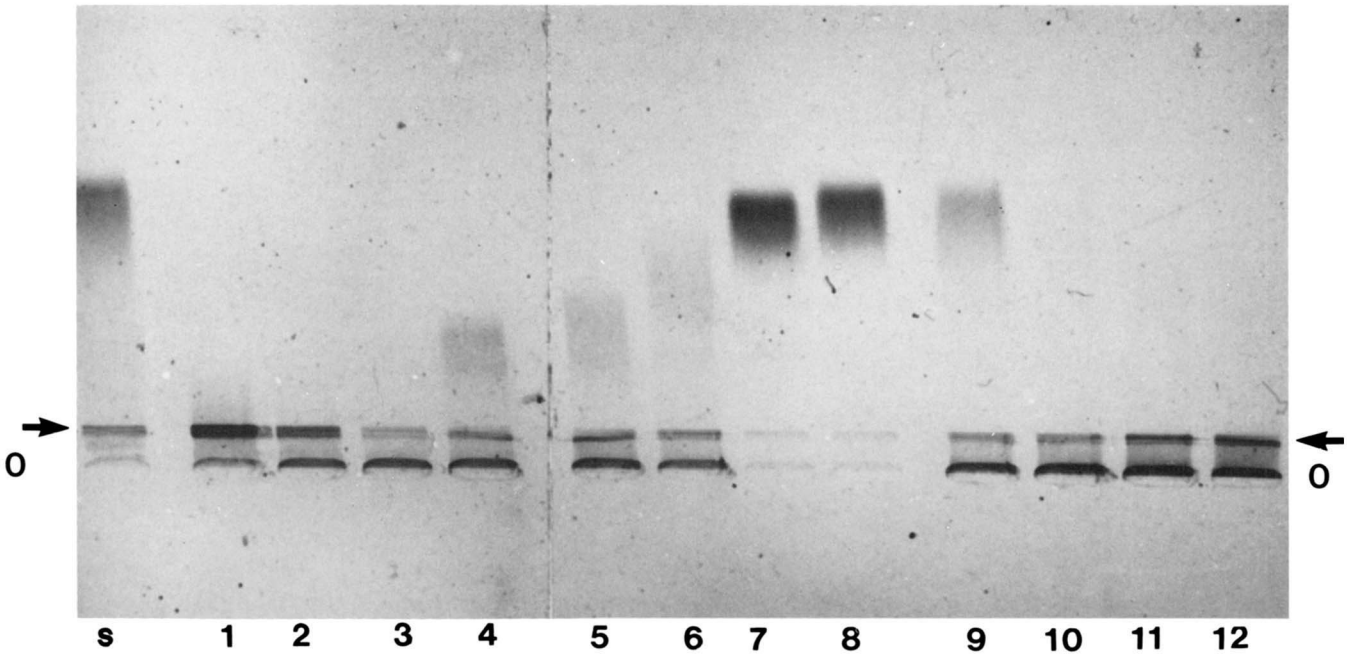


Fig. 1. Polyacrylamide gel film (Lipofilm) electrophoresis of mouse serum (S) and lipoprotein fractions (1–12) isolated by density gradient ultracentrifugation. The densities of the gradient fractions are given in Table 3. Samples of serum or of the various fractions were prestained with Sudan black. The arrows indicate the junction of the 2 and 3% gels; 0 indicates the origin.

first was VLDL-like and was found at the junction of the 2 and 3% gels, while the second had migrated substantially farther into the running gel and to a position typical of HDL. No material with mobility akin to LDL was detected.

Characterization of serum lipoproteins

Analytical ultracentrifugation

The levels of the various classes of lipoproteins, as measured in one of two pools originating from 15 mice, are shown in **Table 2.** Our mouse sera were characterized by high levels of HDL (~500 mg/dl), which represented an average of 83% of the total lipoproteins of $d < 1.21$ g/ml. Material with F in the range ascribed to HDL_2 was resolved as the main subfraction (about 71%), HDL_3-like material accounting for the remainder. The lower limit of the distribution was around 8.6 and the F value of the HDL peak was 4.2; this peak was approximately symmetric. In addition, small amounts of lipoproteins (≤20 mg/dl) were observed in the $F_{1.21}$ 9–20 interval. Levels of total VLDL (~40 mg/dl) and LDL (~70 mg/dl) were low, amounting to 6 and 10%, respectively, of the total. No trace of IDL (S_f 12–20) was detectable. From the analytical ultracentrifugal scans taken at d 1.063 g/ml, it was impossible to obtain a well-defined peak for LDL. Rather these lipoproteins occurred chiefly in the $S_f = 0$ region, but were spread over the LDL region in such a manner that the lower density limit of their distribution was around S_f 10. These results illustrate the inadequacy of conventional conditions and density limits when used for the analysis of mouse LDL by analytical ultracentrifugation, and as such are in complete agreement with the original analyses of mouse serum by Mills and Taylaur (30).

TABLE 2. Concentrations of mouse serum lipoproteins determined by analytical ultracentrifugation[a]

S_f 1.063 Range				$F_{1.21}$ Range		
400–100	100–20	20–12	12–0	9–3.5	3.5–0	Total
			mg/dl			
15.6	25.0	0	69.4	378.9	155.5	534.4

[a] Concentrations were determined by analytical ultracentrifugation at a solvent density of 1.063 g/ml for lipoproteins of S_f 0–400 and at 1.21 g/ml for lipoproteins of $F_{1.21}$ 0–9, according to the conditions of Laplaud et al. (15).

Gradient density ultracentrifugation

The density limits of the successive fractions of 1 ml collected over the density gradient (except for fraction 12, 1.5 ml) are taken from the profile established by Chapman et al. (11) for control NaCl–KBr gradients after ultracentrifugation for 48 hr: these values are shown in **Table 3.** Immunological, electrophoretic, chemical, and morphological data on the gradient subfractions follow.

Immunological reactivity. Upon immunoelectrophoresis and immunodiffusion of density gradient fractions against an antiserum to mouse serum (Table 3), lipoproteins of fractions 1–6 (d < 1.085 g/ml) displayed varying degrees of β-reactivity (**Fig. 2**). Precipitin arcs were strongly stained, particularly in fractions 1 and 2. Faint α-reactivity was observed in fractions 5 and 6, with stronger reactions in fractions 7 to 10: indeed, both α- and β-reactivities were detectable in fractions 5 and 6 (Fig. 2) and occasionally in fraction 4. Serum proteins were present in fractions 11 and 12 (d 1.19–1.25 g/ml) and in trace amounts in fraction 10. Lipid staining was very faint in this latter and absent from fractions 11 and 12.

Electrophoretic mobility. Electrophoresis on polyacrylamide gel slabs revealed three main bands of lipoproteins over the density gradient (Fig. 1, Table 3). Based on the characteristic mobilities of the human substances, fractions 1 and 2 behaved as VLDL and fractions 7, 8, and 9 as HDL. Fractions 4 and 5 migrated to a position intermediate between those of VLDL and HDL and in the region to which human LDL moves. In each of the two latter fractions, bands were diffuse and weakly stained. A faintly staining band was also observed in fraction 6 whose mobility was intermediate between those of LDL (fractions 4 and 5) and HDL. Such results were entirely consistent with those obtained above by immunological methods. Fractions 10, 11, and 12 contained small amounts of lipid-staining material, traces of lipid being detected upon chemical analysis (see **Table 4**); such substances may correspond to VHDL.

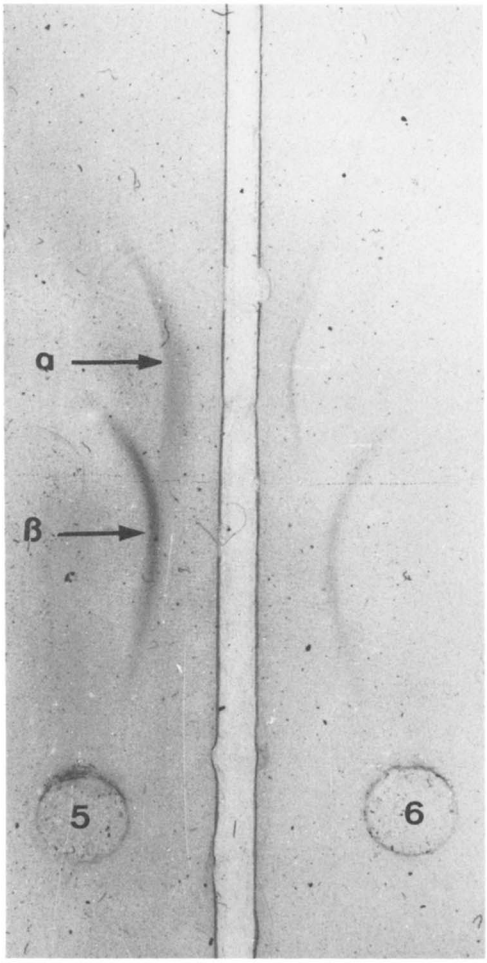
Chemical analysis. The quantitative distribution and mean weight percentage chemical compositions of the gradient fractions are summarized in Tables 3 and 4. Fraction 1 (d < 1.017 g/ml) was rich in lipid, containing only ~8% protein (Table 4). In its high triglyceride content (66%), it clearly resembled VLDL. The composition of fraction 2 (d 1.017–1.023 g/ml) was similar to that of the former, a finding agreeing well with the previous immunological and electrophoretic analyses. Despite minor differences, such as a decrease in triglyceride and an increase of protein content, fraction 3 (d 1.023–1.033 g/ml) was also comparable to VLDL. Fractions 1 and 2 together amounted to some 15–20% of the total substances of d < 1.188 g/ml, whereas fraction 3 was a minor component representing only about 3% (Table 3).

In fractions 4 and 5, triglyceride content was markedly decreased, accounting for about 32 and 23%, respectively, while the proportions of cholesteryl ester and protein were elevated. A substantial increase in phospholipid was also observed. In their chemical composition then, fraction 4 and particularly fraction 5 resembled LDL. However these LDL-like fractions together accounted for only a minor proportion (~6%) of the total mouse lipoproteins. Fraction 6 (d 1.060–1.085 g/ml) exhibited a chemical composition intermediate between those of human LDL and HDL. In its triglyceride content (11%), it was akin to LDL, whereas in protein content (43%) and in its ratio of cholesteryl ester:free cholesterol (8:1), it clearly resembled HDL and, more specifically, HDL_2 (11). Fractions 7 and 8 (d 1.085–1.136 g/ml), as well as fraction 9 (d 1.136–1.163 g/ml), were characterized by a low triglyceride content (3–6%), an elevated ratio of cholesteryl ester: free cholesterol (~9:1), and a high proportion of protein (50–60%). These fractions (i.e., 7, 8, and 9) clearly resembled HDL_3 (see **Table 5**). In the mouse, total HDL (fractions 6–9) accounted for 70% of the lipoproteins of d < 1.188 g/ml and HDL_3-like fractions accounted for 92% of HDL itself. Fraction 10 (d 1.163–1.188 g/ml) was distinct from the HDL_3-like fractions in its diminished ratio of cholesteryl ester:free cholesterol (4:1) and in its low phospholipid content (~9%). This fraction displayed a chemical composition not dissimilar to that of VHDL in man (see fraction IF-d (d 1.153–1.183 g/ml) in ref. 11). Subfractions 11 and 12 (d 1.188–1.253 g/ml) were characterized by high protein contents (99%); nonetheless, they accounted for ~5% of total lipid (Table 3). Comparison of the chemical compositions of the major classes of human serum lipoproteins with the corresponding murine fractions is shown in Table 5; mouse LDL and HDL_3 compositions were calculated on the basis of the sum of the weights of the individual components in the constitutive subfractions (4 and 5 in the case of LDL and 7, 8, and 9 for HDL_3). The resemblance between the VLDL (isolated as d < 1.017 g/ml) of the two species is evident, although the differing amounts of core (apolar) and polar surface components (triglyceride and cholesteryl ester in the former, and protein, free cholesterol, and phospholipid in the latter) are noteworthy. Thus about 73% of the weight is core in mouse VLDL and only ~64% in the human fraction; conversely, 27% of the weight is accounted for by surface constituents in the mouse and 36% in man. These findings suggested that the average particle size of mouse VLDL might be larger than that of their human counterparts (see data on morphometric analysis).

TABLE 3. Profile of lipoprotein subfractions isolated from

	Density Gradient				
	1	2	3	4	5
Density limits (g/ml)[b]	<1.017	1.017–1.023	1.023–1.033	1.033–1.046	1.046–1.060
Immunological activity[c]	β++	β++	β±	β+	β+ α±
Electrophoretic mobility[d]	VLDL	VLDL		LDL	LDL
Serum lipoprotein (mg/dl)[e]	84.2	44.6	20.9	21.9	21.9
Serum lipoprotein protein content (mg/dl)	6.6 ± 1.02	3.6 ± 0.40	2.2 ± 0.41	4.6 ± 0.40	5.7 ± 0.75
Serum lipoprotein lipid content (mg/dl)	77.6 ± 7.30	41.0 ± 1.08	18.6 ± 4.40	17.3 ± 3.69	16.2 ± 1.43
Percent of total lipoprotein of d < 1.188 g/ml	11.0	5.8	2.7	2.9	2.9

[a] Fractions were of ±1 ml except fraction 12 which was removed in a volume of 2.5 ml.
[b] Density limits were taken from a standard curve of density vs. volume derived from control gradients containing only salt solutions.
[c] Immunological reactivity was evaluated by immunoelectrophoresis using antiserum to mouse whole serum. The degree of reactivity was estimated on the basis of the strength of lipid staining of the precipitin arcs: (++) strong reactivity (+) moderate reactivity, and (±) weak reactivity.
[d] Electrophoretic mobility in polyacrylamide gel slabs was compared to that of corresponding lipoproteins of human serum.
[e] The values for each lipoprotein fraction represent the sum of the individual components determined chemically, and are the mean ± SEM of duplicate analyses of each fraction isolated from four density gradients. Each gradient was constructed from a pool of an average of ten mouse sera.
[f] Mainly cholesterol.



Like its VLDL, mouse LDL was substantially poorer in cholesteryl ester and richer in triglyceride that than of man. Other dissimilarities were minor.

Lipid recovery from gradients (fractions 1 to 12, of d < 1.253 g/ml) from four different sera was essentially complete for both cholesteryl ester (97.6 ± 2.5%) and free cholesterol (89.5 ± 7.8), while those of triglyceride (73.8 ± 2.3%, based on a plasma value of 201.5 mg/dl serum; Table 1) and phospholipid (72.5 ± 3.2%) were lower.

Analysis of size distribution and morphology. Electron microscopic examination of three preparations of VLDL of d < 1.017 g/ml revealed particles that were essentially spherical when free standing, but which deformed readily upon contact to form polygonal shapes (**Fig. 3A**). These preparations were highly heterogeneous in size, ranging from about 270 to 750 Å; the mean diameter was 494 ± 7.8 Å. Morphologically, the murine particles could not be distinguished from those of man (11).

Mouse LDL (d 1.023–1.060 g/ml) resembled their human counterparts, appearing as largely spherical particles; the larger particles were subject to considerable deformation in closely packed fields (Fig. 3B). In a representative preparation, most particles (76%) ranged

Fig. 2. Detection of the principle mouse lipoprotein classes by immunoelectrophoresis in agarose gel. Fractions 5 and 6 (d 1.046–1.085 g/ml) (20 μl) were each reacted with a rabbit antiserum to mouse whole serum (central trough, 60 μl) and the slide was stained for lipid with Sudan black.

mouse serum by density gradient ultracentrifugation

Fraction[a]						
6	7	8	9	10	11	12
1.060–1.085	1.085–1.111	1.111–1.136	1.136–1.163	1.163–1.188	1.188–1.214	1.214–1.253
β± α+	α++	α++	α+	α+	serum protein	
HDL	HDL	HDL	HDL			
43.0	265	194	41.3	29.0		
18.9 ± 5.76	130 ± 16.8	99 ± 14.0	25.0 ± 2.70	23.2 ± 2.32		
24.1 ± 6.30	135 ± 13.1	94 ± 14.4	16.3 ± 2.07	5.8 ± 0.18[f]	8.0 ± 1.29[f]	14.8 ± 2.07[f]
5.6	30.4	25.3	5.4	3.9		

from 220 to 280 Å in diameter, with those of 230–255 Å predominating (49.6% of all LDL). The mean diameter was 244 ± 21 Å with a mode of 245 Å.

Characterization of the protein moieties

The presence of a murine counterpart to human apoB (the B_{100} form) (31) was first suggested by the positive reactions given by mouse VLDL and LDL upon immunodiffusion against an antiserum to human apoB (**Fig. 4A and B**); the immunogen employed was human serum LDL of 1.024–1.050 g/ml, some 97–98% of whose protein moiety was comprised of $apoB_{100}$. In both instances, a strong precipitin line was formed between the antiserum and its homologous antigen; this line appeared to cross in a reaction of partial identity with that formed against the murine antigen.

Further evidence for the presence of apoB-like proteins in both murine VLDL (d < 1.017 g/ml) and LDL (d 1.023–1.060 g/ml) was obtained upon electrophoresis of apoVLDL and apoLDL in SDS-polyacrylamide gels (**Fig. 5a and b**); apoB-like polypeptides of high molecular weight (>150,000) predominated. In apoVLDL, the two major bands (denoted 1 and 2) displayed M_r values of 500,000–520,000 and 320,000–350,000, respectively, and were present in a ratio of ~1:3. By contrast, the ratio of these two components in apoLDL was between 2 and 3:1. The human B_{100} protein (31) migrated to the same position as that of murine band 1 in this system; moreover, material eluted electrophoretically (26) from bands 1 and 3 gave a faint precipitin reaction upon immunodiffusion against our antiserum to human apoB (data not shown). These findings strongly suggest that SDS-gel bands 1 and 3 correspond to murine forms of apoB.

Additional apoB-like components were detected in the SDS gel electrophoretic patterns; thus, band 3, a minor form with M_r ~175,000–240,000, was detected in both apoVLDL and apoLDL. Mouse apoLDL was, however, distinguished by the presence of band 1′, with M_r ~ 385,000. No marked qualitative difference in these patterns could be discerned after lipoprotein isolation in the presence of 1 mM PMSF, suggesting that the major forms of apoB do not result from proteolytic degradation in vitro of the higher molecular weight species, i.e., band 1. Nonetheless the band of M_r 175,000–240,000 was quantitatively diminished in VLDL isolated in the presence of PMSF.

In view of the lack of uniformity in nomenclature and in molecular weight described for apoB species in man and in the rat (31–35), we have adopted for present purposes the designation suggested by Sparks and Marsh (33) for the two major forms of (rat) apoB, and shall therefore refer to the murine band 1 species of high M_r as $apoB_H$ and that of lower M_r as $apoB_L$ (i.e., band 2).

High molecular weight forms of apoB were not the exclusive constituents of either apoVLDL or apoLDL in the mouse, as two heavily stained bands of moderate to low apparent M_r (<100,000) were also found in apoVLDL and apoLDL. In VLDL, these displayed M_r

TABLE 4. Chemical composition of lipoprotein subfractions

	Density Gradient				
	1	2	3	4	5
Density limits (g/ml)[b]	<1.017	1.017–1.023	1.023–1.033	1.033–1.046	1.046–1.060
Component (mean weight %)[c]					
Cholesteryl ester[d]	6.3 ± 0.82	7.2 ± 1.37	3.7 ± 1.13	17.9 ± 2.41	25.3 ± 1.77
Free cholesterol	6.2 ± 0.32	5.4 ± 0.24	6.2 ± 0.63	8.5 ± 1.29	6.3 ± 1.22
Triglyceride	66.5 ± 2.10	67.3 ± 2.42	59.6 ± 4.17	32.5 ± 5.95	22.9 ± 2.86
Phospholipid	13.2 ± 0.63	12.0 ± 0.35	13.3 ± 1.39	18.6 ± 3.91	19.6 ± 1.74
Protein	7.8 ± 0.95	8.1 ± 0.94	12.2 ± 3.37	22.4 ± 3.26	25.9 ± 1.59

[a,b] See Table 3.
[c] Values are the means ± SEM of duplicate analyses of each fraction isolated from four density gradients. Each gradient was constructed from a pool of an average of ten mouse sera.
[d] See Table 1.
n.d., not detectable.

values of 70,000–75,000 (band 4) and ~30,000 (band 5) (Fig. 5a). The former band was absent from apoLDL, whose prominent low M_r components were of ~30,000 and <5,000 in size; a further minor band of M_r 53,000 was also detected. It is relevant that estimation of the apparent M_r of proteins of less than ~30,000 in size is fraught with error in gels of low acrylamide monomer concentration.

Further evidence for the presence of a substantial proportion of non-apoB-like apolipoproteins in mouse VLDL and LDL was provided by electrophoresis of TMU-soluble protein in alkaline-urea gels (**Fig. 6a and b**), and by analytical isoelectric focusing of urea-soluble polypeptides of apoVLDL and apoLDL (**Fig. 7a and b**). The identification of certain of these apolipoproteins was facilitated by analysis of apoHDL, in which apoB-like proteins of high M_r were undetectable (Fig. 5c).

Although no reaction could be obtained between mouse HDL and an antiserum to human apoA-I upon immunodiffusion, the SDS gel pattern characteristic of apoHDL was dominated by a single major band, whose leading edge corresponded to an M_r of 25,000–27,000 (Fig. 5c); some diffusely staining material was also identified with M_r of ~9,500 and ~11,500. On the basis of size then, the major polypeptide of murine HDL was a counterpart to human apoA-I, in agreement with earlier reports (8, 10). Data consistent with this conclusion were obtained upon isoelectric focusing. The major protein of mouse HDL_2 and HDL_3 focused as two bands of pH 5.61 and 5.56 (Fig. 7c). The pI values of these two isomorphic forms of apoA-I were indistinguishable from human apoA-I_1 and A-I_2, respectively (Fig. 7d); counterparts to the A-I_3 and A-I_4 isoforms were barely detectable in the rodent lipoproteins. That the proteins with pI 5.61 and 5.56 correspond to the 25,000–27,000 M_r component seen in SDS gels of apoHDL, and in turn to band III in the alkaline-urea gel system (23) (Fig. 6), was demonstrated by elution (see Methods) of the band III polypeptide from such gels and by its subsequent analysis in the SDS-gel and isoelectric focusing systems (data not shown).

Indeed, the band III, apoA-I-like component was the major urea-soluble polypeptide in all mouse lipoprotein density fractions (Fig. 6), accounting for 33–65% of the total densitometrically (**Table 6**). In this system, the relative electrophoretic mobility of mouse apoA-I was in the range 0.24–0.27 (**Table 7**).

At least eight more urea-soluble apolipoproteins were detected in the mouse (Fig. 6); their relative densitometric distributions and electrophoretic mobilities are summarized in Tables 6 and 7, respectively. Among these, three were prominent.

The first, denoted band V in urea gels (Fig. 6), appears to be analogous to the mammalian A-II protein, as upon elution from the latter gel system, it migrated with M_r 8400 in SDS gels but displayed two isoforms with pI values of 4.8 and 5.1 (gels not shown). Human apoA-II, a dimeric protein, displays an M_r of 8700 and pI of 5.0 upon reduction to the monomer (36). By contrast, this apoprotein is most commonly found in monomeric form in the animal kingdom (1). The further characterization of the murine apoA-I and apoA-II-like proteins will form the subject of a subsequent report from this laboratory.

The second, band II, migrated similarly to human apoE, in urea gels (Fig. 6). The presence of an apoE-like polypeptide was also suggested by formation of a precipitin arc between murine VLDL and antiserum to human apoE (Fig. 4c), which fused with that formed between this antiserum and human apoE. In the absence of any detectable spurring of these arcs, it would appear that human and mouse apoE are antigenically similar.

Since the small amounts of band II were not eluted from urea gels (Fig. 6), we are presently unable to assess

isolated from mouse serum by density gradient ultracentrifugation

Fraction[a]						
6	7	8	9	10	11	12
1.060–1.085	1.085–1.111	1.111–1.136	1.136–1.163	1.163–1.188	1.188–1.214	1.214–1.253
20.0 ± 1.69	18.4 ± 0.65	15.4 ± 0.05	12.2 ± 0.74	4.8 ± 0.40	n.d.	n.d.
2.6 ± 0.39	2.0 ± 0.17	1.8 ± 0.17	1.4 ± 0.22	1.2 ± 0.23	n.d.	n.d.
11.1 ± 2.41	3.0 ± 0.17	5.3 ± 0.64	6.3 ± 0.43	5.4 ± 0.85	0.3 ± 0.07	0.1 ± 0.07
23.3 ± 1.67	27.8 ± 0.44	26.0 ± 1.02	19.6 ± 2.36	8.9 ± 1.53	0.5 ± 0.08	0.13 ± 0.006
42.9 ± 3.91	48.8 ± 0.95	51.4 ± 0.86	60.5 ± 2.53	79.6 ± 1.95	99.0 ± 0.08	99.7 ± 0.007

the molecular weight and pI value(s) of this component. Nonetheless, we tentatively suggest that the two polypeptides that focused with pI 5.70–5.80 (Fig. 7) and immediately anodic to apoA-I may correspond to isoforms of murine apoE, although Basu et al. (37) have reported a lower pI (5.4) for this protein. The more basic isoforms of human apoE focus in this region (Fig 7d) (38).

The third, band VIII, migrated as a C protein in alkaline urea gels (Fig. 6) and was the most acidic component (mobility 0.71; Table 6). On this basis, it would be expected to correspond to the acidic apolipoprotein focusing with pI 4.4–4.5 (Fig. 7a–7c). Apolipoproteins focusing in the positions characteristic of apoC-II and the apoC-III and C-III-3 isoforms in man (Fig. 5a), and with pI 4.6–5.1, were thus lacking in mouse.

Finally, some comment on the density distribution of the various urea-soluble apolipoproteins of low M_r is warranted. As illustrated in Fig. 6, VLDL was distinct in lacking band V, the A-II-like protein, which was not only present in all HDL subfractions but also in LDL (Fig. 6b and 6c). By contrast band III, or murine apoA-I, was present throughout the density spectrum; together with band V, it accounted for the major proportion of the low M_r components (51–81% densitometrically in subfractions 2–9; Table 6).

Of the four HDL subfractions (gradient subfractions 6–9, d 1.060–1.163 g/ml), that of d 1.085–1.11 g/ml (Fig. 6e) was distinguished by the unique presence of the band IV polypeptide, and that of d 1.136–1.163 g/ml (Fig. 6g) by the occurrence of band VII (relative mobility 0.57).

The densest material, fraction 10 of d 1.163–1.188 g/ml, a form of VHDL (see Tables 3 and 4) could be differentiated by its possession of essentially all of the aforementioned apolipoprotein bands, i.e., I to VIII.

DISCUSSION

A brief survey of the relevant literature on the plasma lipoprotein profile of the mouse rapidly reveals considerable disparity between observations in different laboratories. As noted earlier, most of the available data concern the electrophoretic profile of mouse lipoproteins, either as components of whole plasma[2] or as isolated fractions (2–7, 9, 10, 39, 40); in only a minority of these studies did the electrophoretic patterns permit clear identification of a fraction migrating with α- or HDL-like mobility as the major lipid carrier in the plasma of *Mus musculus* (4, 7, 9, 10). Without doubt, part of this confusion may have resulted from methodological differences, as well as from variation in the age, sex, and nutritional status of the animals. In addition, dissimilarities in strain may have made an important contribution, as is well illustrated by the contrasting (electrophoretic) pattern seen in the C57BR/cdJ and CBA/J strains by Morrisett et al. (10), and in the vesper mouse (*Calomys lepidus ducillus*), Egyptian spiny mouse (*Acomys cahirinus*), and prairie deer mouse (*Peromyscus maniculatus bairdii*) by Galster and Morrison (5).

For these and other reasons, we were prompted to undertake the present study in the Swiss mouse, in which we found the plasma lipoprotein spectrum to be strikingly dominated by the HDL class. Indeed HDL represented in excess of 70% of the total d < 1.21 g/ml substances when estimated by either the density gradient ultracentrifugal procedure (73%) or by analytical ultracentrifugation (86%). This finding is entirely consistent with measurements made by others when employing either analytical ultracentrifugation (BALB/c

[2] The terms "plasma" and "serum" are used interchangeably.

TABLE 5. Comparison of the chemical composition of the major classes of murine and human serum lipoproteins isolated by density gradient ultracentrifugation

	Mouse VLDL	Human VLDL[a]	Mouse LDL	Human LDL[a]	Mouse HDL			Human HDL	
					HDL_2	HDL_3	Total	HDL_2	HDL_3
Fraction number[b]	1		4 + 5		6	7–9			
Density limits (g/ml)[c]	<1.017	<1.016	1.033–1.060	1.028–1.050	1.060–1.085	1.085–1.163	1.060–1.163	1.066–1.100	1.100–1.153
Component (mean weight %)[d]									
Cholesteryl ester[e]	6.3 ± 0.83	11.7	21.9 ± 1.90	36.8	20.0 ± 2.29	16.6 ± 0.22	17.0 ± 0.07	22.2	17.7
Free cholesterol	6.2 ± 0.33	6.6	7.4 ± 1.18	9.4	2.6 ± 0.39	1.8 ± 0.12	1.9 ± 0.13	5.5	2.6
Triglyceride	66.5 ± 2.10	52.3	28.3 ± 4.07	4.2	11.1 ± 2.41	4.1 ± 0.30	4.6 ± 0.36	4.7	2.9
Phospholipid	13.2 ± 0.63	16.5	19.5 ± 2.56	25.8	23.3 ± 1.67	26.6 ± 0.28	26.7 ± 0.20	29.3	27.8
Protein	7.8 ± 0.95	12.9	24.3 ± 2.02	23.8	42.9 ± 3.91	50.8 ± 0.56	50.8 ± 1.08	38.0	48.8

[a] The compositions of human VLDL, LDL, HDL_2, and HDL_3 are taken from reference 11.
[b,c] See Table 3.
[d] Values are the means ± SEM of duplicate analyses of each fraction from four density gradients. Each gradient was constructed from a pool of an average of ten mouse sera.
[e] See Table 1.

strain) (41) or sequential ultracentrifugal fractionation followed by chemical analysis (Swiss white mouse) (39), BALB/c and C57/BL6 mice (6), or STD:dYY mice (9); but it is in contrast with the observation of Mathur and Spector (8) whose data show VLDL to predominate in Ehrlich ascites tumor plasma when quantitated chemically after prior separation by ultracentrifugal flotation and agarose gel chromatography.

Some discordance also surrounds the question of the degree of homogeneity of murine HDL. For some authors, the HDL class appears rather homogeneous, at least as judged by physicochemical criteria, i.e., flotation rate in the analytical ultracentrifuge of chromatographically isolated subfractions (41), and hydrated density as assessed by rate zonal ultracentrifugation and molecular weight by pore gradient gel electrophoresis (10). Indeed, in these studies, mouse HDL displayed physical properties intermediate between those of human HDL_2 and HDL_3, with molecular weights of 300,000 (41) and 234,000 (10), and hydrated densities in the range 1.098–1.102 g/ml (41) and 1.167 g/ml (10).

In a similar manner, our own analytical ultracentrifugal analyses showed HDL to be distributed approximately symmetrically, the F value of the peak (4.2) being almost at the lower limit of the HDL_2 range (F 3.5–9) and close, therefore, to the upper limit of HDL_3 (i.e., F 3.5). HDL_2 accounted for 71% of HDL by analytical ultracentrifugal analysis, but represented less (about 59%) when estimated by chemical quantitation of gradient subfractions of d 1.060–1.111 g/ml; this minor discrepancy probably arises from differences in the definition adopted for "HDL_2", i.e., HDL_2 is classically isolated (by sequential ultracentrifugation) with a density of 1.063–1.125 g/ml, to which the F range 3.5–9.0 essentially corresponds (14, 16), rather than of d 1.060–1.111 g/ml as performed in the present study (Table 4; subfractions 6 and 7).

Our initial impression of HDL homogeneity was however reassessed upon examination of the chemical composition of HDL subfractions (d 1.060–1.163 g/ml) and of their constituent apolipoproteins. The two subfractions (6 and 7) with densities (1.060–1.085 and 1.085–1.111 g/ml, respectively) approaching that classically attributed to HDL_2 in man (hydrated density 1.09 g/ml (42)) differed in chemical composition (Table 4), and notably in triglyceride content, which was threefold lower in the denser subfraction; in addition, the protein content of subfraction 7 (48.8%) was more akin to those of the denser mouse HDL subfractions of d 1.111–1.136 g/ml, and in turn to that of human HDL_3 (~49%) (11). This prompted us to consider the mouse d 1.060–1.085 g/ml fraction as being structurally and probably metabolically distinct from that of 1.085–1.111 g/ml, and on this basis to represent the murine

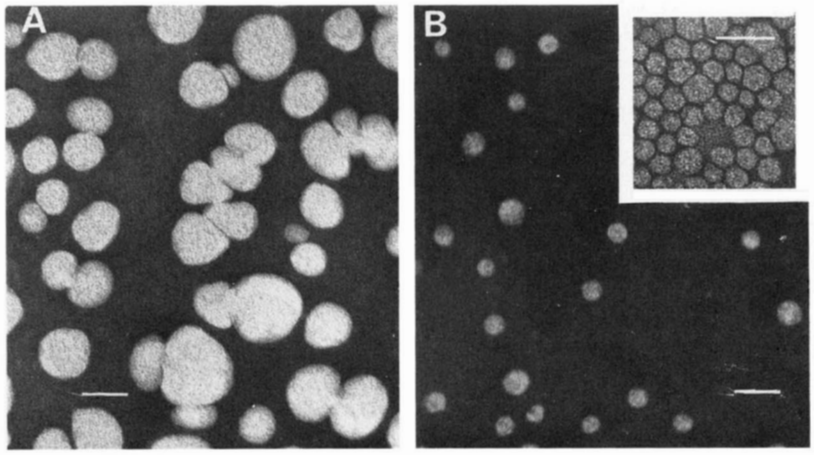


Fig. 3. Electron micrograph of negatively stained murine lipoproteins. A, VLDL, d < 1.017 g/ml; B, LDL of d 1.023–1.060 g/ml. Each horizontal bar represents 500 Å. The insert in B shows the deformation of LDL in packed fields.

counterpart of the human HDL_2 subclass (Table 5). Comparison of the two fractions revealed similar proportions of surface polar components (protein and phospholipid, 66.2 and 67.3% in mouse and human HDL_2, respectively) and of core lipids (cholesteryl ester and triglyceride, 31.1 and 26.9% in mouse and man, respectively). The major distinctions lay in the elevated triglyceride content of the murine lipoprotein, and in its diminished proportion of free cholesterol.

There may be additional grounds on which to distinguish gradient subfractions 6 from 7, since in their apolipoprotein profiles (Fig. 6 and Table 6), the latter was distinguishable by the presence of band IV, which was undetectable in fraction 6. In both subfractions, the mouse apoA-I-like protein predominated in alkaline-urea gels, in which it represented 43–57% of the total densitometrically; only minor amounts of a fast-migrating, apoC-like peptide (band VIII) could be identified (3–4% of total) in each case.

Some evidence for a size and molecular weight difference between subfractions 6 and 7 arose from electrophoresis in polyacrylamide gel gradient slabs (Fig. 1). In this case, fraction 6 migrated to a position intermediate between that of subfractions 5 (LDL of d 1.046–1.060 g/ml) and 7. Indeed, subfractions 7 and 8 (d 1.111–1.136 g/ml) were of similar mobility, both migrating substantially further than subfraction 6, an observation suggesting the latter to be of larger particle size.

Our physical and chemical findings clearly support the notion then that the d 1.060–1.085 g/ml lipoproteins (fraction 6) in the mouse correspond to human

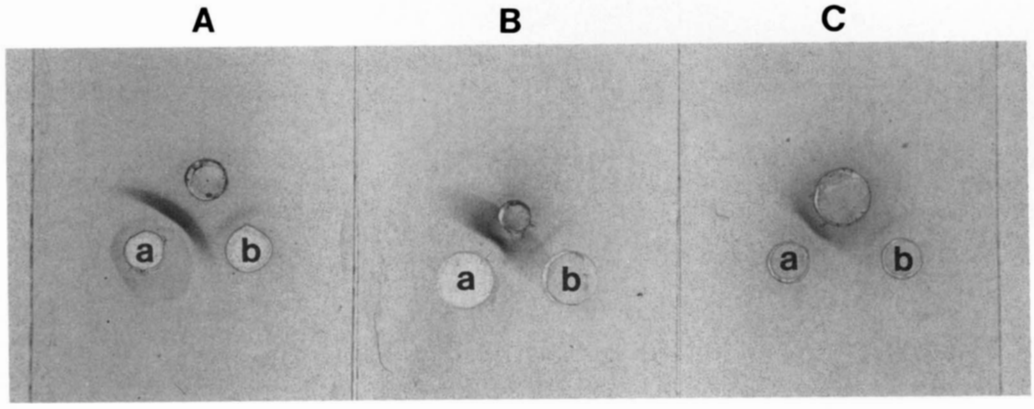


Fig. 4. Immunological reactivity of mouse lipoproteins with antisera to human apolipoproteins upon immunodiffusion. A, Reaction of antiserum to human apoB (8 μl, central well) against (a) human LDL (180 μg protein) and (b) mouse LDL (6 μg protein). B, Reaction of antiserum to human apoB (18 μl, center well) against (a) human VLDL (25 μg protein) and (b) mouse VLDL (2 μg protein). C, Reaction of antiserum to human apoE (20 μl, central well) against (a) human VLDL (25 μg protein) and (b) mouse VLDL (3 μg protein). These slides were stained for protein with Coomassie Brilliant Blue (R 250).

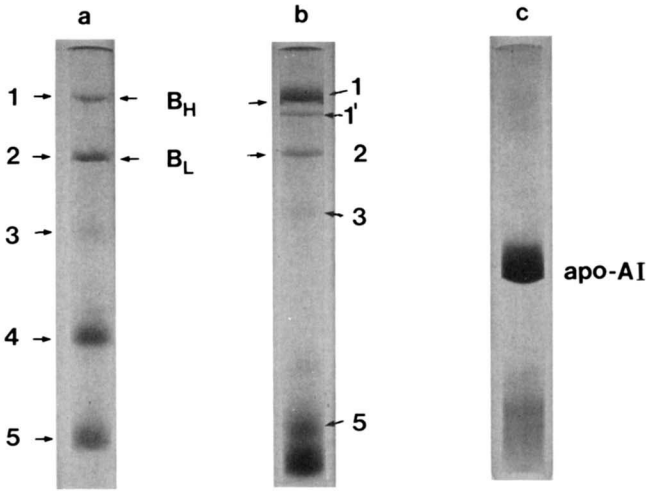


Fig. 5. Electrophoretic patterns in SDS-polyacrylamide gel of the apolipoproteins of mouse serum VLDL, LDL, and HDL isolated by density gradient ultracentrifugation. Samples are (a) apoVLDL, d < 1.017 g/ml; (b) apoLDL, d 1.033–1.060 g/ml; and (c) apoHDL, d 1.060–1.163 g/ml. ApoVLDL and apoLDL were electrophoresed in gels of 4.4% monomer and apoHDL in 10% gels. Gels were stained with Coomassie Brilliant Blue and molecular weights were calculated from a series of purified polymerized marker proteins (see Methods). Bands are numbered in order of diminishing M_r; forms of apoB are denoted as B_H and B_L (33).

HDL_2, of d 1.066–1.100 g/ml when isolated by our gradient procedure (11) (Table 5). A density cutoff of 1.100 or 1.125 g/ml between HDL_2 and HDL_3 in man is clearly inappropriate to the mouse, as is application of the $F_{1.21}$ limits of 0 to 3.5 and 3.5 to 9 to quantitation of murine HDL_2 and HDL_3, respectively, by schlieren analysis. Indeed, a truer reflection of the amount of HDL_2 in murine plasma is that obtained by gradient fractionation, i.e., 43 mg/dl plasma, 6% of total plasma lipoproteins, and 8.7% of total HDL, an observation in sharp contrast to that resulting from analytical ultracentrifugal analysis in which HDL_2 was the major HDL subclass.

In part, this latter observation arises from the fact that the major HDL subfraction detected in our gradient separation was that of d 1.085–1.111 g/ml (i.e., fraction 7); as such it is situated abreast the classic HDL_2/HDL_3 density cutoff, accounting for 54% of total HDL. This finding is entirely consistent with those of Morrisett et al. (10) and Morris and Nelson (41). The resemblance of the chemical composition of subfraction 7 to that of human HDL_3 is remarkable, as is that of subfraction 8 (d 1.111–1.163 g/ml) (Tables 4 and 5). Indeed the composition of the three murine HDL_3 frac-

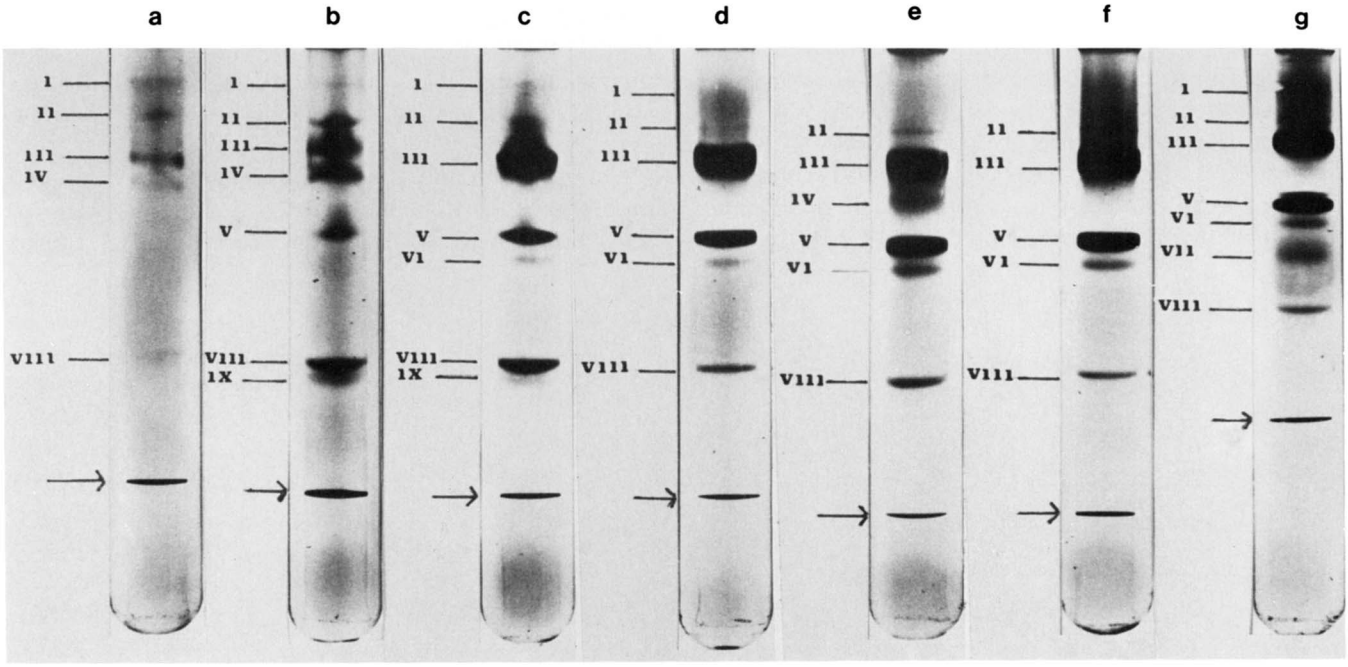


Fig. 6. Electrophoretic patterns of TMU-soluble apolipoproteins of mouse serum lipoproteins separated by density gradient ultracentrifugation. Samples (50 to 100 μg of lipoprotein) are (a) fraction 1, d < 1.017 g/ml; (b) fraction 4, d 1.033–1.046 g/ml; (c) fraction 5, d 1.046–1.060 g/ml; (d) fraction 6, d 1.060–1.085 g/ml; (e) fraction 7, d 1.085–1.110 g/ml; (f) fraction 8, d 1.110–1.136 g/ml; and (g) fraction 9, d 1.136–1.163 g/ml. Apolipoprotein bands are numbered from I to IX in order of increasing mobility relative to dye front (see Tables 6 and 7). Electrophoresis was performed according to the method of Kane (23) in 7.5% polyacrylamide gels containing 8 M urea at pH 8.9. Gels were stained with Coomassie Brilliant Blue. The arrows indicate the dye front in each gel.

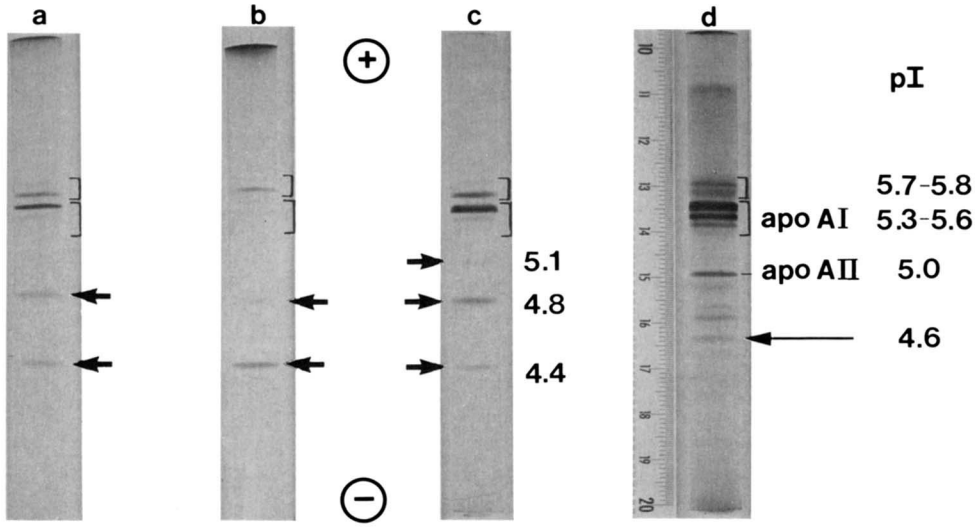


Fig. 7. Analytical isoelectric focusing patterns of mouse apolipoproteins from density gradient subfractions. Samples are (a) LDL, d 1.033–1.060 g/ml; (b) VLDL, d < 1.017 g/ml; (c) HDL_3, d 1.085–1.163 g/ml; and (d) human apoHDL_3, d 1.125–1.21 g/ml; 100–150 μg of urea-soluble protein was applied to each gel. Isoelectric focusing was performed within the pH range 4 to 6.5 (see Methods). Isoelectric points were calculated from a calibration curve constructed from eluates of unstained gels. Coomassie Brilliant Blue was employed for staining protein bands. Human apolipoproteins are identified on the basis of their known pI values.

tions (fractions 7, 8, and 9, d 1.085–1.163 g/ml), calculated as the weighted sum of the individual components, was indistiguishable from its human counterpart (Table 5).

Comparison of the composition of total HDL (d 1.060–1.163 g/ml) in our Swiss mice with that reported in atherosclerosis-resistant and in atherosclerosis-susceptible strains (10) revealed only minor dissimilarities, mainly involving protein (~4.5%) and cholesteryl ester content (3–7%). To the contrary, the HDL of BALB/c and C57BL/6 mice appears quite unique in displaying an unusually elevated phospholipid content (~49–51%) (6).

Although our three HDL_3 subfractions (fractions 7, 8, and 9) displayed mobilities suggestive of similar particle diameter and molecular weight on polyacrylamide gel electrophoresis (Fig. 1), minor differences were evident in the distribution of their TMU-soluble, low M_r polypeptides; these principally concerned the appearance of band IV in fraction 7 and of band VII in subfraction 9 (Fig. 6 and Table 6). They may therefore be metabolically heterogeneous.

The presence of an apoA-I-like component as the major polypeptide of mouse HDL has been suggested on the basis of mobility similar to the human protein in SDS-polyacrylamide gel (10), although it had been

TABLE 6. Percentage distribution of the tetramethylurea-soluble apolipoproteins in the major classes of murine serum lipoproteins

	VLDL	LDL		HDL_2	HDL_3		
Fraction number[a]	1	4	5	6	7	8	9
Density limits (g/ml)[b]	<1.017	1.033–1.046	1.046–1.060	1.060–1.085	1.085–1.111	1.111–1.136	1.136–1.163
Apolipoprotein band number							
I	16.9	5.1	5.9	11.4			20.4
II	25.6	11.4		7.1	12.6	29.1	14.4
III	33.0	32.8	65.1	56.6	42.6	44.8	37.3
IV	8.7	14.8			10.6		
V		18.1	15.9	18.9	23.4	18.3	17.8
VI			2.1	2.8	7.2	5.1	3.3
VII							5.0
VIII	16.3	14.5	1.0	3.2	3.6	2.7	1.8
IX		3.3	8.0				

[a,b] See Table 3. Values represent the % densitometric area of each stained apolipoprotein band and are taken from scans of two 7.5% (w/v) polyacrylamide gels of each lipoprotein fraction; gels were stained with Coomassie Brilliant Blue and scanned at 550 nm.

TABLE 7. Electrophoretic mobilities of the tetramethylurea-soluble apolipoproteins of murine serum lipoproteins

	VLDL (2)	LDL (2)	HDL_2 (2)	HDL_3 (3)
Fraction number[a]	1	4 and 5	6	7, 8, and 9
Density limits (g/ml)[b]	<1.017	1.033–1.060	1.060–1.085	1.085–1.163
Apolipoprotein band number				
I	0.09	0.08	0.09	0.10
II	0.16	0.17	0.17	0.18 ± 0.006
III	0.26	0.24	0.27	0.26 ± 0.007
IV	0.33	0.31		0.31
V		0.44	0.43	0.43
VI		0.49	0.47	0.48 ± 0.008
VII				0.57 ± 0.005
VIII	0.71	0.71	0.72	0.71 ± 0.007
IX		0.77		

[a,b] See Table 3. Electrophoretic mobility is expressed as the ratio of the distance of migration of the individual apolipoprotein to that of the dye front. Mobility values are means ± SEM for the number of preparations given in parentheses. Samples were electrophoresed as indicated in Fig. 3. Soluble apolipoproteins are denoted by their corresponding band number in the stained gel, as shown in Fig. 3.

alluded to earlier (8) in the HDL of ascites plasma. We have confirmed and extended these findings. This protein was akin to human apoA-I in electrophoretic mobility in alkaline urea gels, in its distribution among the HDL subfractions, and in molecular weight (M_r 25,000–27,000); in addition, the two major isomorphic forms displayed pI values (5.61 and 5.56) resembling those of human and rat apoA-I (43–45). Nonetheless, mouse HDL did not cross-react immunologically with an antiserum to the human protein; to our knowledge, an immunological cross-reactivity of rat and human apoA-I has not been reported. Chicken and bovine ApoA-I also fail to react with the homologous human protein (46).

A second major protein of murine HDL may be analogous to the mammalian A-II polypeptide. Identified as band V in the alkaline urea system, this component was of low M_r (8400), thereby resembling the monomeric form of apoA-II typically seen in most mammals (1). Upon isoelectric focusing, band V was resolved into two components with pI values (4.8 and 5.1) resembling the human protein (pI ~5.0) (36). The further characterization of this protein is required for confirmation of these findings; an eventual polymorphism of apoA-II may be of particular interest.

We concur with other investigators in detecting a murine counterpart to rat and human apoE (8, 37, 38, 47, 48).

The possession of an HDL whose modal density is less than that typical of man (14, 16, 42) is a characteristic common to the mouse and rat (49–51). A further resemblance between the plasma lipoprotein profile of *M. musculus* and *Rattus norvegicus* concerns the presence in the mouse of α-reacting lipoproteins in the density range normally attributed to low density substances (i.e., d < 1.063 g/ml). Thus, weak α-reactivity was detected immunologically in subfraction 5 of d 1.046–1.060 g/ml. Such lipoproteins have typically been defined as HDL_1, and have been detected and isolated by ultracentrifugal or electrophoretic procedures in diverse mammals such as rat (48, 52), dog (53), and man (54). In the rat, HDL_1 displays a hydrated density of 1.054 g/ml, contains primarily apoE (>60% of its protein moiety) together with A-I, A-IV, and C peptides, and may be purified by Pevikon-block electrophoresis (48) and density gradient or rate zonal ultracentrifugation (52). In contrast, density gradient ultracentrifugation did not resolve murine HDL_1 as a single lipoprotein species, since the d 1.046–1.060 g/ml fraction contained both α- and β-reacting substances (Table 3). Furthermore, subfraction 5 was seen on pore gradient electrophoresis (Fig. 1) as a diffuse band with LDL-like mobility, thereby indicating its size to be more comparable with that of an LDL particle (~200 Å) than of an HDL (≤100 Å). In chemical composition, subfraction 5 was distinctly different from rat HDL_1 in its more than tenfold excess of triglyceride (52) and lower phospholipid and protein contents (48, 52) (Table 4).

Nonetheless, apoA-I (band III) represented 65% densitometrically of the TMU-soluble apolipoproteins in fraction 5; the A-II-like protein (band V) and the component with apoE-like mobility (band II) were also de-

tected, together with the acidic apoC-like peptide (band VIII). Subfraction 5 is normally however only a minor component of the murine lipid transport system, amounting to ~3% of the d < 1.188 g/ml and 34% of the d 1.023–1.060 g/ml lipoproteins, respectively. Rat HDL_1, to the contrary, may attain 50% of the lipoprotein protein in the d 1.02–1.063 g/ml fraction (55), although this appears subject to nutritional status.

From these findings, it is evident that the tacit use of the 1.063 g/ml density limit as the cutoff point for LDL from HDL is entirely inappropriate in the mouse. Results from density gradient and rate zonal ultracentrifugal analyses (10) are in complete agreement in this regard. Similar comments apply to the rat (30, 49, 50, 55). Unfortunately, the 1.063 g/ml density cutoff has been used in all of the reported studies of murine LDL (6, 8, 9, 30, 39, 40).

The choice of a density interval over which to isolate LDL containing almost exclusively apoB in the mouse is additionally complicated by the presence of apoA-I in subfraction 4 (d 1.033–1.046 g/ml). ApoA-I was confirmed as a constituent of LDL of d 1.033–1.060 g/ml on the basis of its pI values upon isoelectric focusing and its M_r in SDS-gels (band 5, M_r ~ 30,000). Nonetheless, the high molecular weight form of murine apoB, i.e., $apoB_H$, was found by SDS-polyacrylamide gel electrophoresis to be a prominent apoprotein of the d 1.023–1.060 g/ml fraction, and its presence was confirmed by the immunological cross-reactivity of murine LDL with antiserum to human apoB. Other forms of apoB of lower M_r, denoted as band 1′ and as band 2 in SDS-gels, were also found; the latter form, i.e., $apoB_L$, predominated in VLDL (Fig. 5). From these data, we conclude that density gradient ultracentrifugation is inadequate for the separation of apoB-containing particles from those containing primarily apoA-I in the d 1.006–1.063 g/ml interval in the mouse, and suggest that preparative electrophoresis on starch block (56) be used for this purpose.

The low density subfractions 3, 4, and 5 (d 1.023–1.060 g/ml) were uniformly richer in triglyceride and poorer in cholesteryl ester than human LDL. The proportions of core lipids in the mouse fractions (~50% in fractions 4 and 5 and ~75% in fraction 3) are considerably greater than in their human counterparts (~41%; Table 5), suggesting a larger average particle size and volume in the former in order to accommodate the additional hydrophobic material. Such was the case, negative stain electron microscopy revealing an average particle size of 244 Å as compared to 220 Å for human LDL (57), and thus a 19.7% increase in volume. Conversely, the proportions of surface constituents (protein and phospholipid) were only slightly lower in the murine fractions (4 and 5, 41% and 46.6%, respectively) as compared to human LDL (49.6%; Table 5).

Considered as a whole, murine "LDL" of d 1.023–1.060 g/ml represented only about 9% of the total, a proportion similar to that found in a mixture of serum from BALB/c and C57BL/6 mice (~13%) (6). Absolute "LDL" concentrations (65–70 mg/dl) were similar to those of Hsu, Ghanta, and Hiramoto (6) (range 78–103 mg/dl), but superior to those detected by Mills and Taylaur (30) (29 mg/dl) in an undetermined strain.

VLDL accounted for ~12% of d < 1.188 g/ml lipoproteins when determined by chemical analysis after gradient separation, but rather less (~6%) in the serum pool analyzed by analytical ultracentrifugation; actual levels were 84 and 41 mg/dl serum, respectively. This discrepancy may result from differences in lipoprotein levels between individual animals, always a cause of concern when one is obliged to study mixed sera as a result of the small blood volume of individuals. It may also be at least partially due to technical difficulties in accurately quantitating a spectrum of highly heterogeneous particles by schlieren analysis. Thus, mouse VLDL ranged from 270 to 750 Å in diameter, with a mean of 494 Å; as such, they are larger and more heterogeneous than those of normolipidemic males of similar density (<1.017 g/ml) (mean 349 Å; range 200–615 Å) (11). Murine VLDL levels have been variously reported as 13 mg/dl (as S_f 20–400) (30) and 91–128 mg/dl serum (BALB/c and C57BL/6) (6), and marked variations clearly exist between different strains on the same diet (C57BR/cdJ and CBA/J (10); genetically obese ob/ob and mixed hetero- and homo-zygotic lean mice (7)). VLDL concentrations also appear to be age-dependent, since serum triglycerides decrease with increasing age on normal diets in C57BL/6 (6), C57BR/cdJ and CBA/J mice (10). Triglyceride levels (214 mg/dl serum) in our Swiss mice and those in an atherosclerosis-resistant strain (CBA/J) (100–200 mg/dl) (10) were alike, but elevated in comparison to BALB/c, C57BL/6J, and NZB mice (range 35–113 mg/dl) and to atherosclerosis-susceptible animals (C57BR/cdJ) (10). In contrast, serum cholesterol concentrations are rather uniform in all the strains examined to date (range approximately 50 to 110 mg/dl) (6, 7, 10, and the present study), with the possible exception of STD:ddY mice (162 mg/dl) (9).

The chemical compositions of murine VLDL, and of that of the VLDL-like intermediate lipoproteins (fraction 2; Table 3) of d 1.017–1.023 g/ml, were distinct from human VLDL (Table 5) in their higher core lipid content (CE and TG, 72.7% and 64.0% in murine and human VLDL, respectively), and consequently in a reduced proportion of surface constituents (protein, PL

and FC, 27.2 and 36%, respectively). As might be predicted on this basis and noted earlier, the average particle size and volume of the murine particles were superior to those of man. The mouse VLDL isolated by Hsu et al. (6) was substantially richer in phospholipid (35–47% by weight) as compared to those described herein (13.2%).

In addition to apoE, whose presence in mouse VLDL has been described previously (37) and confirmed immunologically in the present study, at least four additional apolipoproteins were present as judged by electrophoretic techniques; these were two putative forms of apoB, designated $apoB_H$ and $apoB_L$ (ratio ~1:3), and proteins of M_r 70,000–75,000 and of ~30,000. The latter, as judged by pI and mobility in alkaline-urea gels, corresponds to murine apoA-I. Indeed, apoA-I (band III) was the predominant TMU-soluble non-apoB component in apoVLDL (Table 6). As in LDL, a partial immunological identity was seen between apoB in human and mouse VLDL (Fig. 4). The low M_r form ($apoB_L$) (33) of mouse apoB (apparent M_r 320,000–350,000) was a major protein of apoVLDL, a finding akin to that in the rat, which displays a lower apparent molecular weight (~240,000) (32, 34), is present in plasma VLDL (32, 34), and is synthesized in both liver and intestine (35). We are therefore led to suggest that some aspects of intestinal and hepatic lipoprotein biosynthesis and catabolism are alike in these two rodents, and in particular, the metabolism of remnants of chylomicrons and hepatic VLDL containing $apoB_L$ and apoE (35, 58). This species resemblance may extend to the metabolism of esterified cholesterol, since the distinct fatty acid distributions of mouse VLDL, LDL, and HDL cholesteryl esters (8, 21) considered together with the preferential enrichment of these lipoproteins in triglyceride at the expense of cholesteryl ester, suggests that cholesteryl ester transfer protein activity may be low in the mouse as in the rat (59).

In conclusion, the major qualitative and quantitative characteristics of the lipid transport system in the Swiss mouse, *Mus musculus,* are presently delineated and reveal several characteristics that are common to the rat, but which contrast with those of other rodents such as the guinea pig (*Cavia porcellus*) (1) and Mongolian gerbil (*Meriones unguiculatus*) (60).

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