The lipid transport system in the mouse, *Mus musculus:* isolation and characterization of apolipoproteins B, A-I, A-II, and C-III

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Abstract Four of the principle apolipoproteins of murine serum have been isolated and characterized. On the basis of their physicochemical properties, they are homologous with the human and rat apoA-I, A-II, B, and C-III. The group of apolipoproteins of middle to low molecular weight, *i.e.*, A-I, A-II and C-III, were separated from the protein moiety of high density lipoproteins (HDL) by gel filtration chromatography, followed by electrophoresis in alkaline-urea polyacrylamide gel with electrophoretic elution. Murine apoA-I, the major protein of HDL (60–80%) displayed an M_r of ~27,000, and was polymorphic (four prominent isoproteins with isoelectric points in the range of pH 5.5–5.7). The amino acid profiles of mouse, rat, and human apoA-I generally resembled each other, the former being distinguished by a content of one isoleucine residue per mole. Amino terminal sequence analysis revealed marked homology between the mouse, rat, dog, and human proteins; mouse and rat apoA-I differed at residues 9 and 18 with potential dissimilarities at residues 5 and 15, while the murine and canine sequences were distinct at residues 6, 9, 13, 15, and 30. Apolipoprotein A-II was a monomer, exhibiting an M_r ~ 11,000 in SDS gels; in addition, it was polymorphic (three apparent isoproteins with pI in the pH range 5.05–5.2), and resembled its human and rat counterparts in amino acid composition. ApoC-III, an acidic peptide of pI 4.74 and of M_r ~ 9,600, possessed an amino acid composition very like that of the homologous human and rat proteins. The homology of mouse apoC-III with the human protein was confirmed by NH_2-terminal sequence analysis, which revealed identical amino acids in six positions (1, 2, 4, 8, 9, and 13). As shown earlier (Camus et al. 1983. *J. Lipid Res.* **24:** 1210–1228), two forms of immunologically reacting apoB predominated in mouse VLDL and LDL. After isolation of these lipoproteins in the presence of 1 mM PMSF, the apparent sizes of the high and low M_r forms, $apoB_H$ and $apoB_L$, were in the ranges ~400,000–530,000 and ~250,000–280,000, respectively, according to the SDS gel system. We observed that inclusion of 1 mM PMSF was essential to retard degradation of the high M_r form $apoB_H$. The murine B proteins were isolated from apoVLDL and apoLDL by gel filtration chromatography on Sephadex G150 in anionic detergent, and displayed apparent M_r values of 460,000 ($apoB_H$) and 250,000 ($apoB_L$) in 3% SDS gels. Only minor dissimilarities were evident between the amino acid profiles of the murine B protein fraction, the human B100 protein, and the rat B protein of M_r 330,000. These findings extend basic knowledge of the major circulating lipid carriers in the mouse, and should permit further development of its use as an animal model for study of the hormonal, genetic, and nutritional modulation of lipid transport.—**Forgez, P., M. J. Chapman, S. C. Rall, Jr., and M-C. Camus.** The lipid transport system in the mouse. *Mus musculus:* isolation and characterization of apolipoproteins B, A-I, A-II, and C-III. *J. Lipid Res.* 1984. **25:** 954–966.

Supplementary key words VLDL • LDL • HDL • gel filtration chromatography • isoelectric focusing • amino acid sequence

The mouse, *Mus musculus,* has for some time been considered an appropriate animal model for studies of a wide variety of normal and pathological processes, and of the effects of genetic, nutritional, and hormonal factors upon them (2–4). However, little attention has been focused on the lipid transport system of this rodent, although several investigators have documented an association between circulating lipid and lipoprotein levels and genetic parameters (4–6). Indeed, it now

Abbreviations: VLDL, very low density lipoproteins, of density as defined; LDL, low density lipoproteins, of density as defined; HDL, high density lipoproteins, of density as defined; PMSF, phenylmethylsulfonyl fluoride; apo, apolipoprotein; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; pI, isoelectric point; HEPES, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid. The nomenclature applied to the murine serum apolipoproteins studied herein, i.e., A-I, A-II, B, and C-III, is that classically employed for their human counterparts, and recently summarized by Alaupovic (1). Analogy has been established on the basis of several criteria which include partial sequence analysis, amino acid composition, molecular weight, isoelectric point, polymorphism, electrophoretic mobility, and quantitative distribution among lipoprotein density classes.

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appears that there are distinct differences in the genetic susceptibility of certain inbred strains, notably the C57BR/cdj and CBA/J, to the dietary induction of atherosclerosis and hyperlipoproteinemia (3, 4). The existence of such strains, together with the earlier established obese and diabetic mutant lines such as the C57 Bl/6J-ob/ob and C57 BL/KsJ-db/db (7), has rapidly brought the mouse to the forefront of lipoprotein research, making it a highly suitable species for investigation of the genetic modulation of lipid transport.

The need for more detailed information on the plasma lipoproteins and apolipoproteins in *M. musculus* has been recognized by several investigators including ourselves (4, 8–13), resulting in the recent documentation of the qualitative and quantitative aspects of the murine lipoprotein distribution in random-bred Swiss mice (12, 13). As in man and a wide range of animal species (10), these murine lipoproteins are characterized by the presence of specialized protein components, available evidence indicating that counterparts to the human A-I, A-II, B, and E apolipoproteins exist in the mouse (4, 9, 11–14). These apoproteins are fundamental in determining the metabolic fate of the parent lipoprotein particle, possessing such biological activities as the ability to bind specifically to cellular lipoprotein receptors (apoB, E) (15), or to activate certain lipolytic enzymes (apoA-I, C-I, and C-II) (16). By contrast, the function of apoA-II remains to be determined.

In the present study, we describe the isolation and principal physicochemical characteristics of four major apolipoproteins of murine serum, apoA-I, apoA-II, apoB, and apoC-III, and compare them to their counterparts in rat and man.

MATERIALS

Animals and diets

Animals used were randomly bred mice of the Swiss OF1 (Iffa Credo) strain, between 6 and 48 weeks of age. The mice were maintained at ambient temperature (22° ± 1°C) with a 12-hr light cycle. They were fed a control diet containing 1364.8 Joules/100 g, whose chief components (% wt) were described earlier (13).

Blood samples

Blood from unanesthetized animals, in either the fasted or fed state, was withdrawn by retro-orbital venous plexus puncture with a narrow bore Pasteur pipette. Serum was separated by low speed centrifugation at 4°C and antibacterial agents were added (0.001% w/v sodium merthiolate and 0.01% w/v sodium azide); in addition, the proteolytic inhibitor PMSF was added to a final concentration of 1 mM unless otherwise noted. The serum was stored frozen for periods up to 1 month before lipoprotein isolation.

PREPARATIVE METHODS

Lipoprotein isolation and delipidation

Lipoproteins were isolated by sequential ultracentrifugal flotation according to Havel, Eder, and Bragdon (17) in a Beckman L5-50 ultracentrifuge employing a Beckman 50Ti rotor. All centrifugations were performed at 50,000 rpm (226,000 g_{av}) and at 10°C. The choice of the density intervals for isolation of the major lipoprotein classes, i.e., VLDL, LDL, and HDL, was based on our preceding study of the distribution of the serum lipoproteins in random bred Swiss mice (13). The interval chosen for LDL, i.e., d 1.017–1.060 g/ml, was used in order to increase the amount of apoB available for isolation. However, as our previous studies have shown (13), triglyceride-rich lipoproteins containing apoB are present over the density range 1.017–1.033 g/ml in the mouse. Such particles will therefore contribute to the "LDL" fraction isolated herein.

VLDL were separated as lipoproteins of d < 1.017 g/ml and LDL were separated in the density interval 1.017–1.060 g/ml, following ultracentrifugation for 24 hr under the conditions described above. HDL, d 1.060–1.21 g/ml, were isolated under similar ultracentrifugal conditions to VLDL and LDL, with the exception that centrifugation was performed for 48 hr. All lipoproteins were washed at their higher limiting density by an additional centrifugation under the same conditions in two to three times their volume of the appropriate NaCl/KBr solution. Each lipoprotein fraction was exhaustively dialyzed against a solution containing 5 mM NH_4HCO_3, 0.05 M NaCl, 0.04% EDTA, 0.01% NaN_3, and 0.001% sodium merthiolate, pH 7.4. Lipoprotein fractions were lyophilized before delipidation.

VLDL and LDL were delipidated at 4°C with a mixture of ethanol–diethyl ether (peroxide-free) 3:1 (v/v) according to Brown, Levy, and Fredrickson (18). HDL were delipidated at −10°C by use of successive mixtures of methanol–chloroform 2:3 (v/v) and 3:2 (v/v) as described by Olofsson, McConathy, and Alaupovic (19).

Apolipoprotein isolation

Apolipoproteins A-I, A-II, and C-III. These apolipoproteins were isolated from murine HDL by gel filtration chromatography followed by electrophoresis in alkaline-urea polyacrylamide gel. ApoHDL (~20 mg) solubilized in 6 M urea (extra pure; Merck) and 2 N acetic acid were loaded onto a Sephadex G-100 column, (Wright, 3.2 × 90 cm), equilibrated with 2 N acetic acid. The column

effluent was monitored continuously at 280 nm with a UV-1 detector, (Pharmacia Fine Chemicals) and fractions of 3 ml were collected on an Isco model 1850 fraction collector.

ApoHDL were separated into four distinct peaks (**Fig. 1**). The respective fractions were pooled as indicated (peaks I–IV) and dialyzed at 4°C overnight against double-distilled water (5 l) and lyophilized immediately.

Apolipoproteins were then further purified by a modification of the electrophoretic elution procedure of Stephens (20). In order to purify apoA-I, we used the column fractions that were most enriched in this protein, i.e., those representing peak II (Fig. 1). For the isolation of apoA-II and apoC-III, the material separated as peak IV was employed. In the subsequent purification of the apolipoproteins, the alkaline-polyacrylamide disc gel electrophoretic system of Davis (21), as modified by Kane (22), was applied. Typically 200 μg of the lyophilized peak material, solubilized in 200 μl of a solution containing 6 M urea and 20 mM ethylmorpholine (SDS, Vitry-sur-Seine, France) at pH 8.6, was loaded onto the top of each gel (dimensions 0.6 × 10.5 cm long). Electrophoresis was then carried out at 1.5 mA/gel at 15°C.

After migration, one of the gels was rapidly stained in 0.04% Coomassie G250 (Sigma) in 3.5% perchloric acid (23). The band corresponding to the specific apolipoprotein that we wished to isolate was cut out from each of the unstained gels on the basis of its position in the stained gel, which was used as a template. The unstained gel slices were crushed and subsequently loaded into a small plastic column, to which was tied a dialysis bag (Spectrapor, M_r cutoff 3,500) at its lower end. The column was placed into the upper tray of an electrophoresis chamber (model GT14, Hoefer Scientific Instruments, San Francisco, CA). The electrophoretic buffers were similar to those used for alkaline polyacrylamide gel electrophoresis (21, 22). In cases in which the sample was intended for amino acid or sequence analysis, the anodic electrolyte solution contained H_3BO_3 (Merck), instead of glycine, at pH 8.9.

Electrodialysis was then carried out for 4 hr at 4 mA/column and at 15°C. On completion, the dialysis bags were removed, tied off, and immediately and exhaustively dialyzed against a solution containing 5 mM NH_4HCO_3 at 4°C. ApoA-I was also isolated by an alternative procedure whose goal was to avoid the use of concentrated urea solutions, which might lead to carbamylation or other structural modification of this apolipoprotein. For this purpose, murine apoA-I was isolated from apoHDL by the method of Stephens (20). Four mg of apoHDL, of which 20% had been dansylated,

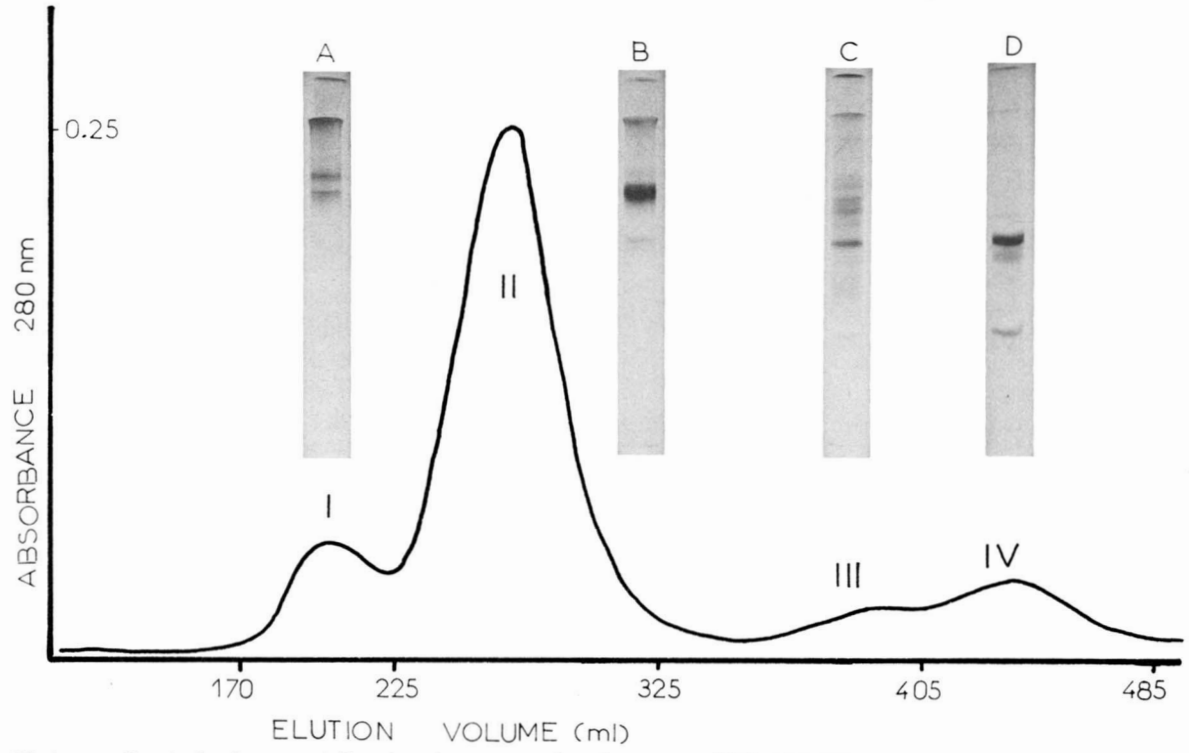

Fig. 1. Elution profile obtained upon gel filtration chromatography of mouse apoHDL (d 1.060–1.21 g/ml; 20 mg protein) on a Sephadex G-100 column (32 × 90 cm) in 2 N acetic acid. Insert, electrophoretic pattern in urea-containing polyacrylamide gels at alkaline pH of material from the pooled fractions of each chromatographic peak. Gel patterns A, B, C, and D correspond to protein eluted in peaks I, II, III, and IV, respectively.

were distributed onto the tops of eighteen SDS-polyacrylamide gels (10% monomer; dimensions 0.6 × 10 cm). After electrophoresis, the band corresponding to apoA-I was localized under U.V. light, and cut out from each gel. Elution by electrodialysis was then performed as above with the exception that the electrolyte buffer was 2.5 mM Tris-glycine (20). The final apoA-I preparation was dialyzed against 5 mM NH_4HCO_3 as described above.

Apolipoprotein B. The mouse apoB-like proteins were purified from VLDL and from LDL by gel filtration chromatography according to Herbert et al. (24) and Chapman and Mills (25), using a Sephadex G 150 column (Wright; 50 × 1.6 cm) equilibrated with a buffer containing 5 mM sodium decyl sulfate, 0.01% (w/v) EDTA and 0.02 M NH_4HCO_3, pH 7.6. ApoLDL and apoVLDL were each solubilized in 2 to 3 ml of this solution, to which was added 1% (w/v) SDS and 1% (w/v) β-mercaptoethanol. The column effluent was monitored at 280 nm using the system described above for HDL apolipoproteins, and collected as described.

ANALYTICAL METHODS

Electrophoretic analyses

Analytical isoelectric focusing was performed in glass tubes in the Hoefer electrophoresis unit (model GT 14) according to Pagnan et al. (26). The electrolyte solutions used were 0.1 M glycine at pH 7.0 (upper buffer) and 0.01 M HEPES at pH 3.5. Focusing was carried out at a constant voltage (400 V) for 5.5 hr at 10°C. Samples from column chromatographic peaks and purified apolipoproteins were examined by electrophoresis in polyacrylamide gels of 3, 4, and 10% monomer concentration using an SDS system previously described by Weber and Osborn (27) or Stephens (20) and modified by Weisgraber et al. (28). Gels were stained with Coomassie Brilliant blue G250 (29). Calibration curves for estimation of molecular weights were constructed from a series of polymerized molecular weight markers ranging in size from 53,000 to 265,000 and from 14,300 to 71,500 daltons (BDH Biochemicals, Poole, U.K.).

Amino acid and sequence analysis

Amino acid analyses were carried out on a Beckman 121 MB analyzer after hydrolysis of samples in 6 N HCl for 20 hr at 110°C in sealed evacuated tubes. Partial sequence analysis was carried out on 35 to 40 nmol of apoA-I and 5 nmol of apoC-III after the addition of polybrene (2 mg in 0.5 ml of H_2O); the protein, dissolved in 0.5 ml of 50% acetic acid, was applied wet to the cup of a Beckman 890 C sequencer. The first cycle was double-coupled; otherwise, a standard 0.1 M Quadrol program (No. 122974) was used. After conversion, phenylthiohydantoin amino acids were identified and quantified on a Beckman 332 High Performance liquid chromatograph, equipped with a CR1A integrator-recorder. The chromatography was developed using the procedure of Somack (30).

Protein determination

Lipoprotein protein and purified apolipoproteins were quantitated by the procedure of Lowry et al. (31) using bovine serum albumin (Sigma) as the working standard.

RESULTS

HDL apolipoproteins

The elution profile obtained upon gel filtration chromatography of murine apoHDL in 2 M acetic acid, the initial step in our fractionation of HDL apolipoproteins, is shown in Fig. 1. Material in peak I corresponded to protein aggregates, and mainly of apoE- and apoA-I-like proteins (bands II and III respectively; Fig. 6, ref. 13), as judged by their respective electrophoretic mobilities (0.18 and 0.24) relative to the dye front, upon electrophoresis in alkaline-urea polyacrylamide gel (Fig. 1, inset gel A). Peak II, the major component of apoHDL (~60–80% of total protein) was constituted primarily of the mouse apoA-I-like protein as shown by alkaline gel electrophoresis (Fig. 1, inset gel B; relative mobility 0.24); traces of apolipoproteins with mobilities of 0.38 and 0.18 were also detectable. Peak III material was composed of a mixture of at least eight components as determined by disc gel electrophoresis (Fig. 1, inset gel C), with relative mobilities of 0.07, 0.18, 0.25, 0.3, 0.32, 0.37, 0.43, and 0.67. However, an apoA-II-like protein (band V, Fig. 6; ref. 13) (mobility 0.37) predominated; this protein was also preponderant in peak IV (mobility 0.39), together with an acidic peptide of high apoC-like mobility (0.67; Fig. 1, inset gel D), and a diffuse minor component with mobility ~ 0.43.

Final purification of the mouse apoA-I-like component was effected from material contained in peak II and achieved by electrophoresis with subsequent elution in alkaline-urea gels (see Methods).

Apolipoprotein A-I

The murine A-I-like protein purified in this manner and eluted electrophoretically exhibited the following physicochemical characteristics. By electrophoresis in SDS-polyacrylamide gels of 10% monomer concentration (27), the M_r of this protein was in the range 27,000 to 30,000 daltons (**Fig. 2,** gel C); some load dependency of the M_r value was noted. Upon analytical isoelectric

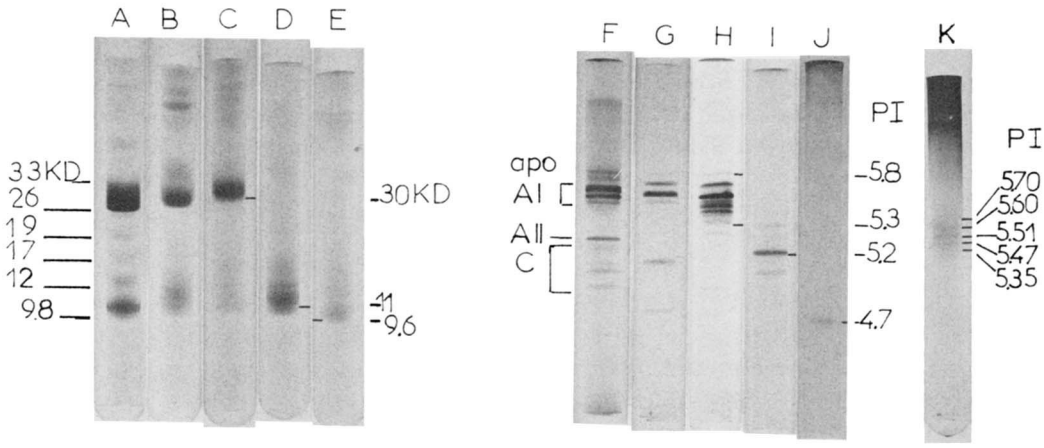

Fig. 2. Comparison of the electrophoretic patterns of mouse apoA-I purified by gel filtration chromatography and electrophoresis in urea gels (gels C and H), apoA-II (gels D and I), and apoC-III (gels E and J) with that of mouse apoHDL (gels B and G) and human apoHDL$_3$ (gels A and F). In gel K, mouse apoA-I was purified from SDS-polyacrylamide gels according to Stephens (21). At left, gels A to E: patterns in 10% SDS-polyacrylamide gels for determination of molecular weight. At right, gels F to K: analytical isoelectric focusing patterns in the pH range 4 to 6.5. Fifty to 100 μg of protein was applied in each case. All gels were stained with Coomassie Brilliant Blue R 250.

focusing in the pH range 4–6.5 (Fig. 2, gel H), the major A-I band had a pI value of 5.61 ± 0.14 (n = 9); up to three additional bands were also prominent with isoelectric points of 5.69 ± 0.13, 5.54 ± 0.14, and 5.49 ± 0.14. (n = 9). Above and below these major components, we consistently detected single weak bands with pI of 5.83 ± 0.1 and 5.37 ± 0.17, respectively. Only about three of these bands were seen in mouse apoHDL (Fig. 2, gel G), and these had pI values in the range ~ 5.8–5.5. The three to four additional, more acidic bands in our purified murine apoA-I may have arisen during the preparative urea gel electrophoretic procedure by carbamylation of lysine residues in apoA-I isomorphs (24), or alternatively by processes of oxidation or partial deamidation. The latter phenomenon has indeed been suggested to occur during isolation of human apoA-I (32, 33) in which case more acidic apoA-I isoforms became prominent.

To evaluate this question further, we isolated murine apoA-I by the procedure of Stephens (20), using SDS-polyacrylamide gels and avoiding urea solutions. In this case, the focusing pattern was essentially identical to that described above. Six isomorphic forms were detected (Fig. 2, gel K); three major forms predominated with pI values of 5.60, 5.51, and 5.47, respectively. The minor bands displayed pI values of 5.70, 5.35, and 5.28. That the more acidic forms do not arise from carbamylation is consistent with the absence of carbamyl-lysine residues upon amino acid analysis. Comparison of the focusing patterns of apoA-I separated by the two procedures revealed only minor differences (Fig. 2, gels H and K), indicating that if any artefactual forms are created, they arise in both isolation methods. It is noteworthy that the focusing pattern of dansylated apoA-I was diffuse and superimposable on the above patterns, and the bands were in the pH range 5.30–5.70. We are led to suggest that this polymorphism may be due, at least in part, to isomorphic forms present in such small amount as to be undetectable in holo-apoHDL. This type of phenomenon has been noted during the purification of vervet (34) and marmoset (35) apoA-I.

The amino acid composition of murine apoA-I resembled that of the equivalent protein in man (36) and in the rat (37) (**Table 1**). Thus mouse apoA-I contained elevated amounts of lysine, arginine, aspartic, and glutamic acids (including that present in the amide forms, i.e., asparagine and glutamine), serine, alanine, valine, and leucine. Indeed, these same amino acids were present in large amounts in the rat and human proteins. Minor differences were evident between the compositions of this protein in the two rodents.

There were also dissimilarities between the murine and human A-I proteins as is evident from Table 1. Furthermore, with the exception of its leucine content, the amino acid profiles of mouse and human apoA-I appeared to be more closely related than those of the two rodent proteins. Mouse apoA-I contained one residue of isoleucine/mol distinguishing it not only from its homologue in man but also in rat, whose A-I had an elevated content (4.6 mol/mol) of this hydrophobic residue.

The results of our partial sequence determination on 40 nmol of apoA-I from *M. musculus* are summarized in **Fig. 3** for six amino acids, i.e., aspartic acid, valine, glutamine, alanine, lysine, and tyrosine, while the amino acid sequence to the thirtieth residue is depicted in

TABLE 1. Amino acid composition of apolipoproteins A-I, A-II, and C-III from mouse, rat, and man

Amino Acid	Apolipoprotein A-I			Apolipoprotein A-II			Apolipoprotein C-III		
	Mouse[a]	Rat[b]	Man[c]	Mouse[d]	Rat[e]	Man[f]	Mouse[g]	Rat[h]	Man[i]
Lys	16.8 ± 0.4	20.7	21	5.2	3.8	9	3.8 ± 0.5	5.5	6
His	4.8 ± 0.4	4.7	5	1.0	0	0	1.2 ± 0.4	0	1
Arg	15.7 ± 0.5	12.2	16	1.3	3.1	0	2.0 ± 0.3	1.6	2
Asp	25.2 ± 0.5	31.4	21	5.4	6.9	3	7.8 ± 0.8	7.1	7
Thr	13.1 ± 0.2	11.2	10	5.1	5.4	6	4.0 ± 0.3	5.5	5
Ser	16.7 ± 1.0	10.1	15	7.8	4.6	6	10.1 ± 0.6	7.9	11
Glu	48.6 ± 0.9	41.9	46	13.8	16.1	16	13.1 ± 0.4	11.8	10
Pro	9.5 ± 0.04	6.7	10	3.6	4.6	4	3.0 ± 0.9	2.4	2
Gly	9.8 ± 1.8	11.2	10	5.6	2.3	3	9.5 ± 0.7	7.9	3
Ala	14.6 ± 0.3	18.1	19	6.7	9.2	5	5.8 ± 0.2	4.7	10
Val	15.8 ± 1.0	12.6	13	3.4	2.3	6	4.5 ± 0.3	2.4	6
Met	4.6 ± 0.1	5.6	3	2.5	2.3	1	1.3 ± 0.05	3.2	2
Iso	1.0 ± 0.2	4.6	0	1.3	0.8	1	1.9 ± 0.1	0.8	0
Leu	31.6 ± 1.0	30.1	37	6.5	8.5	8	5.5 ± 0.6	8.7	5
Tyr	5.2 ± 0.2	4.9	7	2.8	3.1	4	1.9 ± 0.2	2.4	2
Phe	5.4 ± 0.2	6.7	6	4.4	3.8	4	3.0 ± 0.4	3.2	4
Cys*	0.4 ± 0.2	1.1	0	0	0	1	0.5	0	0
Tryptophan	n.d.	2.2	4	n.d.	0	0	n.d.	3.2	4

[a] Values are expressed as moles of amino acid/mole of A-I protein and are means ± SD of triplicate analyses on each of four separate preparations of apoA-I. Calculation was based on the assumption that mouse apoA-I contains 239 residues (tryptophan excluded) as in man.
[b] Assumed number of residues 236 (37).
[c] Assumed number of residues 243 (36).
[d] Values are expressed as moles of amino acid/mole of apoA-II protein of two separate preparations of apoA-II. Calculation was based on the assumption that murine apoA-II contains 77 residues as in man.
[e] Assumed number of residues 77 (38).
[f] Assumed number of residues 77 (39).
[g] Values are expressed as moles of amino acid per mole of C-III protein and are means ± SD of triplicate analyses on each of three separate preparations of apoC-III. Calculation was based on the assumption that mouse apoC-III contains 79 residues as in man (40).
[h] Assumed number of residues 79 (38).
[i] Assumed number of residues 79 (40).
n.d., Not determined; *, determined as cysteic acid (41).

Table 2 and compared to that for the homologous protein from the rat, dog, and man. The sequence in the rat and murine proteins differed at position 9, which was arginine in the former and lysine in the latter species, and at position 18, which was valine in the mouse but unidentified in the rat. However, it is known that this amino acid cannot be valine, as this amino acid was tested for in the rat sequence work (42). Whereas in rat, at positions 5 and 15 no identification was made, these residues are serine and asparagine, respectively, in mouse. In the rat study, in view of the amino acids that were tested for, both residues 5 and 15 could be the same as those in mouse.

Apolipoprotein A-II

Apolipoproteins A-II and C-III were purified by a similar strategy as that applied to apoA-I above, with the qualification that peak IV material from the gel filtration step on Sephadex G-100 (Fig. 1) was taken for this purpose.

The murine apoA-II-like protein (mobility 0.39; Fig. 1, gel D) presented the following physicochemical characteristics. Upon molecular weight determination by electrophoresis in SDS-polyacrylamide gel (27), our system gave an M_r in the range 11,000–12,000 (Fig. 2, gel D); an equivalent band was detected in mouse holo-apoHDL (Fig. 2, gel B) with M_r of 11,000. By analytical isoelectric focusing in the pH range 4 to 6.5, purified murine apoA-II displayed one major band with pI 5.22 ± 0.15; two minor components were also detectable (Fig. 2, gel I), exhibiting slightly more acidic and slightly more basic isoelectric points (5.06 ± 0.03 and 5.33 ± 0.09, respectively (n = 3)). By comparison with holo-apoHDL (Fig. 2, gel G), only one band was present in this region of the gel, with a pI (5.14 ± 0.14; n = 4) differing slightly from that of the major component in purified A-II (pI ~ 5.22).

In general, the amino acid compositions of the rodent and human A-II proteins were alike. However the mouse A-II protein possessed 1 residue of histidine, whereas this amino acid is absent from the homologous rat and human proteins. In addition, murine apoA-II contained one residue of arginine, whereas the rat protein contains three residues; in human A-II, arginine is absent. The human protein was characterized by the presence of one residue of cysteine; indeed, the A-II protein of only one other species has been found to contain cysteine, to exist as a disulfide-linked dimeric

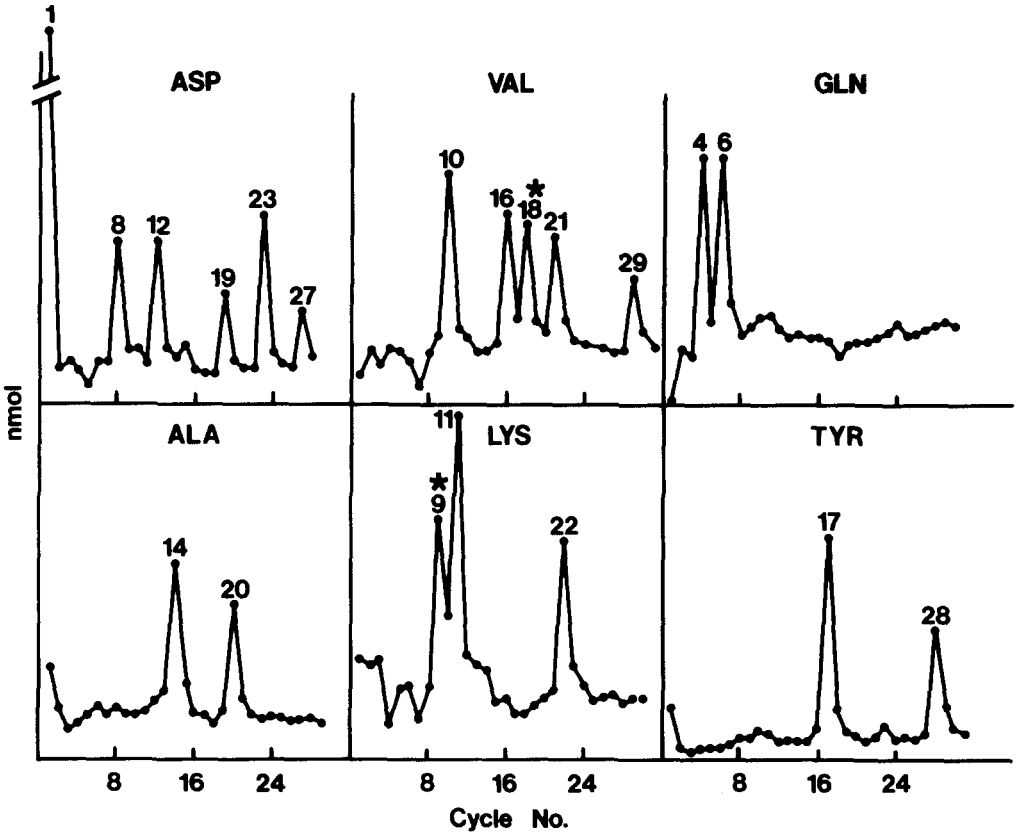

Fig. 3. NH_2-terminal sequence analysis of apolipoprotein A-I isolated from murine plasma HDL. Data are plotted as nmol amino acid (in the PTH form) recovered at each degradative cycle, and are shown for six representative amino acids; these include the two residues (valine in position 18 and lysine in position 9) at which differences with the rat sequence were found (see Table 2), and are denoted by asterisks. Degradation was performed on 40 nmol of apoA-I and the initial yield of Asp was 13 nmol.

protein, and that is the chimpanzee, *Pan troglodytes* (44). As in the case of apoA-I, the observed polymorphism of apoA-II does not appear to arise from carbamylation, inasmuch as carbamyl-lysine was absent upon amino acid analysis.

Apolipoprotein C-III

By electrophoresis of murine apoHDL in alkaline-urea polyacrylamide gels, only a single prominent component was visible in the zone to which the human C-II and C-III peptides typically migrate (not shown); this band displayed a relative electrophoretic mobility of ~0.7. The murine apoC-like protein was subsequently purified by the same approach as described above for apoA-I and apoA-II. This procedure yielded a peptide with M_r ~ 9,600 on SDS-gels of 10% monomer (27) (Fig. 2, gel E), and with an isoelectric point of 4.74 (Fig. 2, gel J). The amino acid composition of the murine

TABLE 2. NH_2-terminal sequence of apolipoprotein A-I from the mouse, rat, dog, and man

Species	Residue Number																												
	. . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 . . . . . 25 30																												
Mouse[a]	D	E	P		Q	S	Q	W	D	K	V	K	D	F	A	N	V	Y	V	D	A	V	K	D	S	G	R	D	Y	V	S
Rat[b]	D	E	P		Q	X	Q	W	D	R	V	K	D	F	A	X	V	Y	X	D	A	V									
Dog[b]	D	E	P		Q	S	P	W	D	R	V	K	D	L	A	T	V	Y	V	D	A	V	K	D	S	G	R	D	Y	V	A
Man[b]	D	E	P	P	Q	S	P	W	D	R	V	K	D	L	A	T	V	Y	V	D	V	L	K	D	S	G	R	D	Y	V	S

Homologous regions in murine, rat, canine, and human apoA-I are indicated by rectangles, taking into account the deletion of proline at residue 4 in the animal apoA-I.

[a] Determined by sequence analysis of purified apoA-I from murine apoHDL.

[b] The rat, dog, and human sequences are taken from references 42, 43, and 36, respectively.

TABLE 3. NH_2-terminal sequence of apolipoprotein C-III from mouse and man

Species	Residue Number																				
	1						5								10						
Mouse[a]	Ser	-	Glu	-	Val	-	Glu	-	Gly	-	Ser	-	Leu	-	Leu	-	Gly	-	Ser	-	Val	-	Gln	-
Man[b]	Ser	-	Glu	-	Ala	-	Glu	-	Asp	-	Ala	-	Ser	-	Leu	-	Leu	-	Ser	-	Phe	-	Met	-	Gln	-

Homologous regions in murine and human apoC-III are indicated by rectangles.
[a] Determined by sequence analysis of purified apoC-III from murine apoHDL.
[b] The human sequence is taken from reference 40.

apoC-III-like component indeed resembled that of the rat and human C-III peptides (Table 1). By comparison with human apoC-III, the mouse protein possessed two residues of isoleucine, and the rat one. This amino acid is characteristically absent from human C-III. It is also of interest that while rat C-III was devoid of histidine, this amino acid was present in both the human and murine C-III peptides.

The results of partial sequence analysis to the thirteenth residue of the murine C-III peptide are presented in **Table 3,** and reveal that six residues, in positions 1, 2, 4, 8, 9, and 13 are identical with those in the homologous human protein (40). By contrast, murine apoC-III shared only one residue in common with human apoC-I (45) and two residues with human apoC-II (46). It is of note that the amino acid substitutions in human C-III could have arisen from single base changes in the respective codons.

VLDL and LDL apolipoproteins

Our earlier studies on the protein moieties of VLDL and LDL by immunodiffusion revealed a positive precipitin reaction against an antiserum to human apoB in each case (13), suggesting the presence of a murine counterpart to the human B-100 protein (47). In the present investigations, the SDS-polyacrylamide gel patterns obtained from VLDL and LDL, isolated ultracentrifugally in the presence of the proteolytic inhibitor PMSF, indicated that the mouse possessed two major B proteins (**Fig. 4,** gels B and C). The largest, denoted $apoB_H$ in accordance with the nomenclature proposed for the rat B proteins (48), displayed an apparent M_r of 534,000 ± 34,000 in 3% monomer SDS gels (20, 28). The B-100 protein of human LDL presented a similar apparent M_r (513,000 ± 23,000) when examined in the same gel system under similar conditions (Fig. 4, gel A). The smaller murine B protein, $apoB_L$, was of M_r 278,000 ± 16,000; applying the centile nomenclature of Kane, Hardman, and Paulus (47), this protein bears a 52:100 relationship with the high M_r form, suggesting that it may be analogous to the human B-48 protein. The relative amounts of $apoB_H$ and $apoB_L$ in murine VLDL differed from those in LDL (13), a finding confirmed in the present study (Fig. 4, gels B and C).

When examined in SDS-polyacrylamide gels of 4% monomer concentration, the two major murine B proteins exhibited apparent M_r values of 395,000 ± 14,000 and 251,000 ± 9,000, while human apoB-100 displayed an M_r of 366,000 ± 16,000. Disparities similar to those evident between the M_r values displayed by murine $apoB_H$ in the 3 and 4% gel systems (i.e., 536,000 and 395,000 in 3 and 4% monomer, respectively) have also been observed in the apparent M_r of the marmoset B-100 protein (35). Thus, there is clearly a tendency for the apparent M_r of the largest B protein to increase in our SDS system upon passing from gels of higher (4%) to lower (3%) acrylamide monomer concentration.

In 4% SDS-gels of murine apoVLDL and apoLDL, we detected three additional bands of low M_r, estimated to be of 35,000, 28,000, and 19,000 (data not shown). Traces of the largest of these proteins were visible at

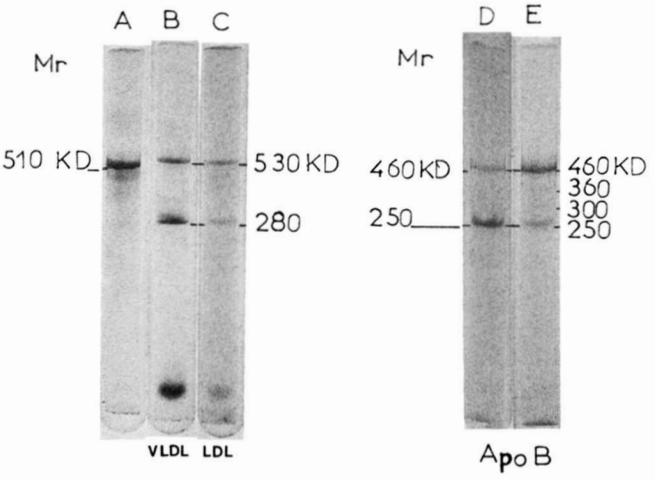

Fig. 4. Electrophoretic patterns in SDS-polyacrylamide gel of mouse apoVLDL, apoLDL, and the corresponding column fraction I obtained by gel filtration chromatography in cationic detergent on a Sephadex G-150 column. Patterns: A, human apoLDL (d 1.024–1.050 g/ml) isolated in the absence of PMSF; B, mouse apoVLDL (d <1.017 g/ml) VLDL isolated ultracentrifugally in the presence of 1 mM PMSF; C, mouse apoLDL (d 1.017–1.060 g/ml) isolated from serum in the presence of 1 mM PMSF; D, fraction I, containing murine apoB-like proteins, isolated from apoVLDL (gel B) by gel filtration chromatography in buffers lacking PMSF; E, fraction I, containing murine apoB-like proteins chromatographically isolated from apoLDL in buffers lacking PMSF. The starting material, serum LDL, was separated ultracentrifugally in the presence of PMSF. The acrylamide monomer concentration in gels A to E was 3%; 20–40 μg of protein was applied. Gels were stained with Coomassie Brilliant Blue R 250.

the bottom of our 3% gels, and notably in VLDL (Fig. 4, gels B and C). The intermediate band of M_r 28,000 was more prominent in apoLDL, and was absent or present in trace amounts in apoVLDL.

We next attempted to fractionate the murine B proteins in sodium decyl sulfate by gel filtration chromatography on a Sephadex G-150 column (18, 24, 25). The elution profiles obtained at 280 nm from apoVLDL (d < 1.017 g/ml) and apoLDL (d 1.017–1.060 g/ml) each presented three peaks. The first, peak I, eluted in the excluded volume, the second at a retention volume of 60 ml (peak II), and the third (peak III) at the total volume (200 ml) of the column. In both apoVLDL and apoLDL, peak I represented ~40% of the total protein applied, while peak II material accounted for some 40% of apoVLDL and 30% of apoLDL, and peak III accounted for 20% and 30% of apoVLDL and apoLDL, respectively.

By SDS gel electrophoresis in 3% gels, peak I from apoLDL contained two major proteins, the larger of apparent M_r 460,000, the smaller of M_r 250,000 (Fig. 4, gel E); these components appear to correspond to $apoB_H$ and to $apoB_L$, respectively.

While $apoB_L$ of M_r 250,000 was a major component in peak I from apoVLDL (Fig. 4, gel D), the proportion of this component was lower in the corresponding fraction from apoLDL (Fig. 4, gel E); the M_r 460,000 protein ($apoB_H$) was the major protein in gel E; the relative proportions of $apoB_H$ and $apoB_L$ seen in apoVLDL (Fig. 4, gel B) and apoLDL (Fig. 4, gel C) were therefore conserved in the chromatographically isolated fraction. The B protein pattern in apoLDL was distinguished by the presence of two additional minor bands, of M_r 360,000 and 300,000, respectively, that were between $apoB_H$ and $apoB_L$.

These two minor bands were not, however, identifiable in apoVLDL or apoLDL when SDS-gel electrophoresis was performed rapidly after lipoprotein isolation and dialysis. By contrast, when the native lipoproteins were stored at 4°C for ~1 week, or when PMSF was not present during lipoprotein isolation, such bands appeared, and, in particular, a component of M_r ~ 330,000 (gels not shown). These observations suggest that the trace components of M_r 360,000 and 300,000 seen in murine apoB, separated chromatographically, may have arisen as a result of slight degradation occurring during the chromatographic procedure.

Peak II material from the G-150 column contained two bands, the first with M_r 250,000 and the second with M_r 36,000 (not shown).

The apoB-like proteins in column peak I from apoVLDL and apoLDL were further characterized by amino acid analysis (**Table 4**). Data are compared to the composition of the human B-100 protein and the rat B

TABLE 4. Amino acid composition of apolipoprotein B from murine, rat, and human plasma lipoproteins

	Murine ApoB[a]		Human B-100[b]		Rat ApoB[c]	
Amino Acid	LDL	VLDL	LDL	VLDL	(1)	(2)
Lys	5.4	5.6	8.0	8.1	7.1	7.5
His	1.5	1.8	2.6	2.6	2.1	2.1
Arg	3.9	4.4	3.4	3.5	4.8	4.8
Asp	10.1	10.0	10.7	10.6	10.9	10.9
Thr	6.3	6.3	6.6	6.6	6.2	5.8
Ser	10.2	9.0	8.6	8.4	7.8	6.8
Glu	13.7	13.4	11.6	11.7	14.0	15.0
Pro	3.8	3.6	3.8	3.8	3.9	3.9
Gly	8.3	8.6	4.7	4.8	6.6	6.4
Ala	7.2	7.5	6.1	6.1	6.5	6.5
Val	5.9	5.9	5.6	5.5	6.4	6.4
Met	1.7	1.7	1.6	1.7	1.8	1.8
Iso	4.4	4.0	6.0	6.1	4.4	3.6
Leu	10.2	10.3	11.8	11.8	10.6	11.6
Tyr	2.8	2.9	3.4	3.3	3.0	3.2
Phe	4.2	3.9	5.1	5.0	4.4	4.3
Cys	0.5	1.0	0.4	0.4	n.d.	n.d.

Data are expressed as mol/100 mol; n.d., not determined.

[a] Values are means from two separate preparations of each sample, each analyzed in duplicate or triplicate. The murine B protein fraction was isolated as peak I upon gel filtration chromatography of either apoVLDL or apoLDL on Sephadex G-150 in cationic detergent (see Methods).

[b] Data on man from ref. 47.

[c] Data on rat apoB are taken from Krishnaiah et al. (50). Components 1 and 2 correspond to the 330,000 complex and the 240,000 peptide of apoB, respectively.

proteins of M_r 330,000 and 240,000, respectively. It is noteworthy that the amino acid profiles of the B protein fraction of murine apoVLDL and of apoLDL closely resembled each other, despite the differing proportions of the various B protein species (Fig. 4, gels D and E). The largest disparity was in serine content (1.2 mol %).

DISCUSSION

The continuing development of the mouse as an animal model for investigation of the effect of nutritional and genetic factors on lipid transport demands that the structural characteristics of the circulating apolipoproteins be determined, and that their structure be related, wherever possible, to a functional role. To this end, we describe the physicochemical characteristics of four of the major serum apolipoproteins in random-bred Swiss mice, which are analogous to those of the human and rat A-I, A-II, C-III, and B proteins.

The group of apolipoproteins of intermediate to low molecular weight, namely A-I, A-II, and C-III, were purified from HDL, taking advantage of the fact that this lipoprotein class predominates in *M. musculus*. Indeed, calculation shows that apoA-I levels (~100–150 mg/dl) are in a range similar to those of man (50), and moreover, that it is the most abundant apolipoprotein in the Swiss mouse. The physical properties, chemical composition, and N-terminal sequence of the murine protein clearly establish its homology with human and rat apoA-I (10, 36–38). Thus the molecular weight of murine apoA-I (27,000–30,000) resembled that of the corresponding protein (~27,000) in other mammalian species (10), including both *Rattus norvegicus* (37) and man (36). In addition, it was polymorphic, presenting one major (pI 5.61) and several minor isoforms (up to five; pI range of the three principal components, 5.49–5.69); such behavior is equally typical of the rat (four to six isoforms with pI in the range 5.46–5.82) (51) and human proteins (about four isoforms, pI range 5.3–5.6 (52, 53)). In an attempt to clarify the origin(s) of such polymorphism, murine apoA-I was purified by an alternative procedure (20). Similar findings were, however, made and carbamylation was effectively excluded. The possibility that one of the more basic isoforms may correspond to murine proapoA-I as detected by Miller et al. (54) cannot be excluded. The isoelectric points of the murine, rat, and human A-I isoforms resemble each other and at the same time overlap to some degree with those of the corresponding E proteins (pI ranges 5.7–5.8 in mouse (12, 13), 5.31–5.46 in rat (51), and ~5.4–6.1 in man (55)). In chemical composition, mouse apoA-I was closely related to both its rat and human analogues, although certain aspects of its amino acid profile were more akin to those displayed by the human rather than by the rat protein (notably isoleucine content, Table 1). The homology between these different apoA-I's was confirmed upon partial sequence analysis of the mouse protein (Table 2). A distinct difference from its counterpart in rat was found at residue 9 (lysine in mouse and arginine in rat), with potential dissimilarities at residue 5 (serine in mouse, and possibly serine or threonine in rat (42)), residue 15 (asparagine in mouse, unidentified in rat, and threonine in all other species examined to date (10)), and residue 18 (valine in mouse and man but not apparently valine in the rat (42)). Comparison of the rodent and canine proteins (43) revealed additional differences, notably at residues 6 and 13, and at residue 30 in the mouse (Table 2). Interestingly, the residue mutation at position 9 in the rat and mouse proteins is highly conservative, since it would involve only a single change in a nucleotide base in the respective codons. Indeed, the residue differences between the canine and murine A-I's are related in a similar manner.

Although preliminary studies of the lecithin: cholesterol acyl transferase (LCAT) system in murine plasma have been described (56), a potential role of the A-I protein as an LCAT activator remains to be evaluated.

The second major murine apolipoprotein to be characterized displayed physicochemical properties suggesting its homology with the human and rat A-II proteins. Thus, in amino acid composition, murine apoA-II markedly resembled its counterpart in rat and man; mouse A-II was, however, distinguished by one histidine residue per mole of protein, while both the rat and human apoA-II lacks this amino acid. On the other hand, the presence of the rodent proteins as monomers was reflected in their deficiency in cysteine, the human apolipoprotein existing as a disulfide-linked dimer and containing one such residue per mole (30).

The isoelectric point of the major isoform of mouse apoA-II was 5.22, resembling rather closely that of the human and rat proteins (pI values ~5.1 (52, 53) and 4.83 (57), respectively). Nonetheless, the murine apolipoprotein appeared distinct in exhibiting a polymorphism; we had earlier shown the presence of two isoforms with pI values of 4.8 and 5.1 in band V material eluted from alkaline-urea gels of holo-apoHDL (13). We presently confirmed this finding, since in addition to the major A-II isoprotein, two additional minor components were detected as faint bands (Fig. 2, gel I) with isoelectric points of 5.06 and 5.33. The polymorphism of apolipoprotein A-II in random-bred Swiss mice has been confirmed recently (12, 14), two isoforms being detected. Furthermore, such isoforms correspond precisely to those denoted as alloforms A and B (14) and detected in several strains of inbred mice; the precise pI values

of the latter alloforms have not been reported and therefore limit comparison with the present data. It is particularly noteworthy that human apolipoprotein A-II has been shown to be polymorphic (58), presenting four isoproteins with pI values of 4.31, 4.58, 4.89, and 5.16. Indeed, it will be of interest to determine the molecular basis of the polymorphism in both the human and mouse proteins; at least a partial contribution of deamidation cannot be excluded in the present investigation.

We originally estimated the molecular weight of mouse A-II in SDS-gels as 8400 (13), although a slightly higher value was presently found (~11,000) by the same procedure; such variation (~10–25%) in the apparent size of small proteins has been typically observed in our hands in this system. The monomeric form of the human protein is of similar molecular weight (8700) (39), as is that of rat (57).

During the completion of the present study, we became aware of the data of LeBoeuf and colleages (12, 14) on apolipoproteins A-I and A-II in Swiss mice and in a number of inbred strains. Our results are largely in agreement with the latter (at least as concerns the Swiss strain), with one notable exception. This concerns the proportion of arginine in these proteins, which in our analyses is about two- to fourfold higher than in analyses of the latter authors, and more closely related to the levels of this basic residue in the human and rat proteins (Table 1) (12). No apparent explanation for this discrepancy is evident.

The major murine peptide akin to the C apoproteins in man was a counterpart to apoC-III as judged on the basis of its amino acid composition (Table 1), apparent molecular weight (~9,000), and isoelectric point (4.74). No polymorphism was detectable, although we cannot exclude the possibility that band IX, seen in alkaline-urea gels of mouse apoHDL (13), may not represent an isoprotein. Murine apoC-III was further identified by NH_2-terminal sequence analysis to the thirteenth residue, which revealed sequence homology with human apoC-III in six positions. Clearly, apolipoprotein C-III has undergone considerable evolutionary change, and it will be of interest to compare the present sequence data with comparable information on the C-III peptides of other mammals. In view of the apparently small number of C peptides in mouse, evaluation of the possible metabolic role of this apolipoprotein appears of some importance, particularly with regard to lipolytic mechanisms.

One apolipoprotein with potential receptor binding activity has to date been clearly identified in *M. musculus*, and this is homologous with human and rat apoE (11–13). The presence of a second such apolipoprotein, presenting two major forms and collectively known as apoB, has also been described in this rodent on the basis of immunological and electrophoretic studies of apo-VLDL and apoLDL (12, 13). While it is agreed that two major forms exist as in rat and man (47–49), the apparent M_r values reported for the murine proteins are at variance. Thus LeBoeuf et al. (12), using a 5% SDS-acrylamide system, reported the largest form to have M_r ~ 350,000, with the smaller at 220,000. Our own findings suggest the former ($apoB_H$) to be of M_r 400,000–530,000 and the latter ($apoB_L$) of M_r 250,000–280,000. No immediate explanation is evident to account for these disparities, although it is established that the determination of the M_r of proteins in SDS gel systems tends to be subject to dependency on protein load and acrylamide monomer concentration (35, 47). Minor satellite bands also existed, both groups of investigators suspecting some contribution of proteolysis to the B protein patterns in mouse VLDL and LDL (12, 13). In our studies of the B proteins of a variety of mammalian species, it has become apparent that the mouse B proteins, especially the high M_r form $apoB_H$, are highly susceptible to degradation. We have presently found that such degradation may be significantly retarded by the presence of 1 mM PMSF during lipoprotein isolation. Future investigation of the murine B proteins should therefore be conducted in the presence of effective protease inhibitors.

Purification of the mouse B proteins was accomplished by gel filtration chromatography on Sephadex G-150 in decyl sulfate-containing buffer, although this system did not permit isolation of the $apoB_H$ and the $apoB_L$ polypeptides as separate entities. In contrast, the Sepharose CL-6B system of Sparks and Marsh (48) facilitated separation of their counterparts in the rat. The limited quantities of murine B proteins presently available to us effectively precluded this approach.

Amino acid analysis of the mouse B proteins further confirmed the homology of these polypeptides with their counterparts in man and rat (Table 4), extending our earlier immunological observations (13), and attesting to the wide distribution of B proteins as fundamental components of the lipid transport systems in mammalian species (10).

In summary, the present and earlier (13) studies extend our knowledge of mouse serum lipoproteins and permit further development of *M. musculus* as an experimental animal model for investigation of the effect of genetic, hormonal, and nutritional factors on lipid transport and metabolism. In particular, we now envisage development of immunological assay systems for monitoring apolipoproteins B, A-I, A-II, and C-III levels under various physiological conditions.

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REFERENCES

1. Alaupovic, P. 1980. The concepts, classification systems and nomenclatures of human plasma lipoproteins. *In* Electrophoresis. Vol. I: Lipoproteins. L. A. Lewis and J. J. Opplt, editors. CRC Press, Boca Raton, FL. 27–46.
2. Gill, J. G. 1980. The use of randomly bred and genetically defined animals in biomedical research. *Am. J. Pathol.* **101:** 521–530.
3. Roberts, A., and J. S. Thompson. 1976. Inbred mice and their hybrids as an animal model for atherosclerosis. *Adv. Exp. Med. Biol.* **67:** 313–327.
4. Morrisett, J. D., H-S. Kim, J. R. Patsch, S. K. Datta, and J-J. Trentin. 1982. Genetic susceptibility and resistance to diet-induced atherosclerosis and hyperlipoproteinemia. *Arteriosclerosis.* **2:** 312–324.
5. Weibust, R. S. 1973. Inheritance of plasma cholesterol levels in mice. *Genetics.* **73:** 303–312.
6. Yamamoto, R. S., L. B. Crittenden, L. Sokoloff, and G. E. Jay, Jr. 1963. Genetic variations in plasma lipid content in mice. *J. Lipid Res.* **4:** 413–418.
7. Bray, G. A., and D. A. York. 1979. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol. Rev.* **59:** 719–809.
8. Salmon, D. M. W., and D. A. Hems. 1973. Plasma lipoproteins and the synthesis and turnover of plasma triglyceride in normal and genetically obese mice. *Biochem. J.* **136:** 551–563.
9. Mathur, S., and A. A. Spector. 1976. Characterization of the Ehrlich ascites tumor plasma lipoproteins. *Biochim. Biophys. Acta.* **424:** 45–56.
10. Chapman, M. J. 1980. Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.* **21:** 789–853.
11. Basu, S. K., M. S. Brown, Y. K. Ho, R. J. Havel, and J. L. Goldstein. 1981. Mouse macrophages synthesize and secrete a protein resembling apolipoprotein E. *Proc. Natl. Acad. Sci. USA.* **78:** 7545–7549.
12. LeBoeuf, R. C., D. L. Puppione, V. N. Schumaker, and A. J. Lusis. 1983. Genetic control of lipid transport in mice. I. Structural properties and polymorphisms of plasma lipoproteins. *J. Biol. Chem.* **258:** 5063–5070.
13. Camus, M-C., M. J. Chapman, P. Forgez, and P. M. Laplaud. 1983. Distribution and characterization of the serum lipoproteins and apoproteins in the mouse, *Mus musculus*. *J. Lipid Res.* **24:** 1210–1228.
14. Lusis, A. J., B. A. Taylor, R. W. Wangenstein, and R. C. LeBoeuf. 1983. Genetic control of lipid transport in mice. II. Genes controlling structure of high density lipoproteins. *J. Biol. Chem.* **258:** 5071–5078.
15. Mahley, R. W., and T. L. Innerarity. 1983. Lipoprotein receptors and cholesterol homeostasis. *Biochim. Biophys. Acta.* **737:** 197–222.
16. Smith, L. C., H. J. Pownall, and A. M. Gotto. 1978. The plasma lipoproteins: structure and metabolism. *Annu. Rev. Biochem.* **47:** 751–777.
17. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
18. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies on the proteins of human plasma very low density lipoproteins. *J. Biol. Chem.* **244:** 5687–5694.
19. Olofsson, S-O., W. J. McConathy, and P. Alaupovic. 1978. Isolation and partial characterization of a new acidic apolipoprotein (apolipoprotein F) from high density lipoproteins of human plasma. *Biochemistry.* **17:** 1032–1037.
20. Stephens, R. E. 1975. High resolution preparative SDS-polyacrylamide gel electrophoresis: fluorescent visualization and electrophoretic elution-concentration of protein bands. *Anal. Biochem.* **65:** 369–379.
21. Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. NY Acad. Sci.* **121:** 404–427.
22. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
23. Reisner, A. H., P. Nemes, and C. Bucholtz. 1975. The use of Coomassie Brilliant Blue G250 perchloric acid solution for staining in electrophoresis and isoelectric focusing on polyacrylamide gels. *Anal. Biochem.* **64:** 509–516.
24. Herbert, P. N., R. S. Shulman, R. I. Levy, and D. S. Fredrickson. 1973. Fractionation of the C-apoproteins from human plasma very low density lipoproteins. *J. Biol. Chem.* **248:** 4941–4946.
25. Chapman, M. J., and G. L. Mills. 1977. Characterization of the serum lipoproteins and their apoproteins in hypercholesterolemic guinea pigs. *Biochem. J.* **167:** 9–21.
26. Pagnan, A., R. J. Havel, J. P. Kane, and L. Kotite. 1977. Characterization of human very low density lipoproteins containing two electrophoretic populations: double prebeta lipoproteinemia and primary dysbetalipoproteinemia. *J. Lipid Res.* **18:** 613–622.
27. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
28. Weisgraber, K. H., T. P. Bersot, R. W. Mahley, G. Franceschini, and C. R. Sirtori. 1980. A-I Milano apoprotein. Isolation and characterization of a cysteine-containing variant of the A-I apoprotein from human high density lipoproteins. *J. Clin. Invest.* **66:** 901–907.
29. Karlson, C., H. Dories, J. Ohman, and U. B. Anderson. 1973. Analytical thin-layer gel electrofocusing in polyacrylamide gel. LKB application note no. 75.
30. Somack, R. 1980. Complete phenylthiohydantoin amino acid analysis by high performance liquid chromatography on Ultrasphere octadecyl trimethyloxysilane. *Anal. Biochem.* **104:** 464–468.
31. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. S. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
32. Jackson, R. L., J. D. Morrisett, and A. M. Gotto. 1976. Lipoprotein structure and metabolism. *Physiol. Rev.* **56:** 259–316.
33. Osborne, J. C., Jr., and H. B. Brewer, Jr. 1977. The plasma lipoproteins. *Adv. Protein Chem.* **31:** 253–337.

34. Parks, J. S., and L. L. Rudel. 1979. Isolation and characterization of high density lipoprotein apoproteins in the non-human primate (vervet). *J. Biol. Chem.* **154:** 6616–6723.
35. Forgez, P., M. J. Chapman, and G. L. Mills. 1983. Isolation, characterization and comparative aspects of the major serum apolipoproteins, B-100 and A-I, in the common marmoset, *Callithrix jacchus. Biochim. Biophys. Acta.* **754:** 321–333.
36. Brewer, H. B. Jr., T. Fairwell, A. Larue, R. Ronan, A. Hauser, and T. Bronzert. 1978. The amino acid sequence of human apoA-I, an apolipoprotein isolated from high density lipoproteins. *Biochem. Biophys. Res. Commun.* **80:** 623–630.
37. Swaney, J. B., F. Braithwaite, and H. A. Eder. 1977. Characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry.* **16:** 271–273.
38. Herbert, P. N., H. G. Windmueller, T. P. Bersot, and R. S. Shulman. 1974. Characterization of the rat apolipoproteins. I. The low molecular weight proteins of rat plasma high density lipoproteins. *J. Biol. Chem.* **249:** 5718–5724.
39. Brewer, H. B., Jr., S. E. Lux, R. Ronan, and K. M. John. 1972. Amino acid sequence of human apoLp-Gln-II (apoA-II), an apolipoprotein isolated from the high density lipoprotein complex. *Proc. Natl. Acad. Sci. USA* **69:** 1304–1308.
40. Brewer, H. B., Jr., R. Shulman, P. N. Herbert, R. Ronan, and K. Wehrly. 1974. The complete amino acid sequence of alanine apolipoprotein (apoC-III), an apolipoprotein from human plasma very low density lipoproteins. *J. Biol. Chem.* **249:** 4975–4984.
41. Moore, S. 1963. On the determination of cystine as cysteic acid. *J. Biol. Chem.* **238:** 235–237.
42. Gordon, J. I., D. P. Smith, R. Andy, D. H. Alpers, G. Schonfeld, and A. W. Strauss. 1982. The primary translation product of rat intestinal apolipoprotein A-I mRNA is an unusual preproprotein. *J. Biol. Chem.* **257:** 971–978.
43. Chung, H., A. Randolph, I. Reardon, and R. L. Heinrikson. 1982. The covalent structure of apolipoprotein A-I from canine high density lipoproteins. *J. Biol. Chem.* **257:** 2961–2967.
44. Scanu, A. M., C. Edelstein, and R. H. Wolf. 1974. Chimpanzee (*Pan troglodytes*) serum high density lipoproteins. Isolation and properties of their two major apolipoproteins. *Biochim. Biophys. Acta.* **351:** 341–347.
45. Jackson, R. L., J. T. Sparrow, H. N. Baker, J. D. Morrisett, O. D. Taunton, and A. M. Gotto, Jr. 1974. The primary structure of apolipoprotein-serine. *J. Biol. Chem.* **249:** 5308–5313.
46. Jackson, R. L., H. N. Baker, E. B. Gilliam, and A. M. Gotto, Jr. 1977. Primary structure of very low density apolipoprotein C-II of human plasma. *Proc. Natl. Acad. Sci. USA.* **74:** 1942–1945.
47. Kane, J. P., D. A. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
48. Sparks, C. E., and J. B. Marsh. 1981. Analysis of lipoprotein apoproteins by SDS-gel filtration column chromatography. *J. Lipid Res.* **22:** 514–518.
49. Krishnaiah, K. V., L. F. Walker, J. Borensztajn, G. Schonfeld, and G. S. Getz. 1980. Apolipoprotein B variant derived from rat intestine. *Proc. Natl. Acad. Sci. USA.* **77:** 3806–3810.
50. Karlin, J. B., and A. H. Rubinstein. 1979. Serum lipoprotein quantification by immunochemical methods. *In* The Biochemistry of Atherosclerosis. A. M. Scanu, R. W. Wissler, and G. S. Getz, editors. Marcel Dekker, New York. 189–227.
51. Gidez, L. I., J. B. Swaney, and S. Murnane. 1977. Analysis of rat serum apolipoproteins by isoelectric focusing. I. Studies on the middle molecular weight subunits. *J. Lipid Res.* **18:** 59–68.
52. Nestruck, A. C., G. Suzue, and Y. L. Marcel. 1980. Studies on the polymorphism of human apolipoprotein A-I. *Biochem. Biophys. Acta.* **617:** 110–121.
53. Forgez, P., and M. J. Chapman. 1982. A simplified and efficient procedure for the purification of apolipoprotein A-I from human serum high density lipoprotein-3 by preparative isoelectric focusing on polyacrylamide gel beads. *J. Biochem. Biophys. Methods.* **6:** 283–296.
54. Miller, J. C. E., R. K. Barth, P. H. Shaw, R. W. Elliott, and N. D. Hastie. 1983. Identification of a cDNA clone for mouse apoprotein A-I (apoA-I) and its use in characterization of apoA-I mRNA expression in liver and small intestine. *Proc. Natl. Acad. Sci. USA.* **80:** 1511–1515.
55. Weisgraber, K. H., S. C. Rall, Jr. and R. W. Mahley. 1981. Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apoE isoforms. *J. Biol. Chem.* **256:** 9077–9083.
56. Owen, J. S., V. Ramalho, J. C. M. Costa, and M. P. T. Gillett. 1979. Determination of lecithin:cholesterol acyltransferase in mouse plasma and the influence of mercaptoethanol and sulphydryl blocking agents on its activity. *Comp. Biochem. Physiol.* **63B:** 261–265.
57. Swaney, J. B., and L. I. Gidez. 1977. Analysis of rat serum apolipoproteins by isoelectric focusing. II. Studies on the low molecular weight subunits. *J. Lipid Res.* **18:** 69–76.
58. Schmitz, G., K. Ilsemann, B. Melnik, and G. Assmann. 1983. Isoproteins of human apolipoprotein A-II: isolation and characterization. *J. Lipid Res.* **24:** 1021–1029.