Physicochemical characterization of ten fractions of bovine alpha lipoproteins[1]

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Abstract With the onset of milk production, serum concentrations of alpha lipoproteins in the dairy cow steadily increase, frequently attaining values greater than 1.5 g/dl. Since these lipoproteins comprise a highly polydisperse system, we have carried out studies to explore differences among bovine alpha lipoproteins in the density interval between 1.05 to 1.21 g/ml. Separation into ten fractions was achieved ultracentrifugally in an isopycnic gradient. Agarose gel electrophoresis showed that all but the bottom fraction contained alpha lipoproteins as either the major or sole lipoprotein class. Compositional analyses revealed an increasing percentage of both protein and phospholipid and a decreasing percentage of cholesterol with increasing fraction density. The esterified to unesterified cholesterol ratio ranged from 3 to 8 from the top to the bottom of the gradient. The densities of the particles obtained from the various fractions were calculated both from sedimentation velocity measurements and from compositional analyses. The resulting density values agreed well with the solution densities of these isopycnic gradient fractions. The major apoprotein of each fraction was apoA-I. Combining diffusion coefficient data obtained by intensity fluctuation spectroscopy with sedimentation velocity data, we were able to calculate molecular weights, frictional ratios, and Einstein-Stokes radii for three of the fractions. Results are discussed in terms of previously published data on bovine lipoproteins as well as other mammalian data.—**Puppione, D. L., S. T. Kunitake, M. L. Toomey, E. Loh, and V. N. Schumaker.** Physicochemical characterization of ten fractions of bovine alpha lipoproteins. *J. Lipid Res.* 1982. **23:** 371–379.

Supplementary key words cow • lactation • gestation • inelastic light scattering • HDL

Lactating cows develop a hypercholesterolemia, associated almost exclusively with increasing concentrations of alpha lipoproteins, which can be isolated within the density interval 1.09 to 1.04 g/ml (1, 2). The rise in serum cholesterol begins shortly after parturition, with a maximum occurring during mid-lactation, and then levels return to pre-partum values during the latter stages of lactation (3–5). Serum of the lactating cow is then an excellent source of large quantities of alpha lipoproteins having densities extending over a broad range, from 1.21 to less than 1.04 g/ml (6). The alpha lipoproteins isolated within the low density class have a mean diameter of 15.5 nm (6). By comparison, normal human subjects have smaller alpha lipoproteins with hydrated densities ranging almost exclusively between 1.09 and 1.21 g/ml.

We were interested in studying differences in the physicochemical properties of these bovine alpha lipoproteins. The 1.05–1.21 g/ml density fraction was first isolated, and further separation of the alpha lipoproteins into ten fractions was achieved in an isopycnic gradient following 40 hr of ultracentrifugation. The physicochemical characterization described in this report included detailed compositional studies, agarose electrophoresis, hydrodynamic studies, and calculations of molecular weights, frictional ratios, and Einstein-Stokes radii.

MATERIALS AND METHODS

Isolation of lipoproteins

Separate studies were carried out on sera obtained from seven lactating, non-pregnant cows (a Jersey, a Guernsey, and five Holstein Friesians). Sera and all salt solutions used for ultracentrifugation contained Na_2·EDTA (0.04%), NaN_3 (0.05%), and Gentamycin

Abbreviations: apoA-I, the major apoprotein of high density lipoproteins, HDL, isolated between the density interval 1.063–1.21 g/ml; apoA-IV, a minor HDL apoprotein, having a molecular weight of 46,000; apoB, the major apoprotein of beta-migrating low density lipoprotein, LDL, isolated between the density interval 1.020–1.063 g/ml; apoC, a group of apoproteins with molecular weights between 8,000 and 12,500, present on HDL and triglyceride-rich lipoproteins; apoE, a group of apoproteins present on HDL and triglyceride-rich lipoproteins, also called the "arginine-rich proteins", ARP. Symbols: M, molecular weight; η, viscosity; ϕ, apparent specific volume.

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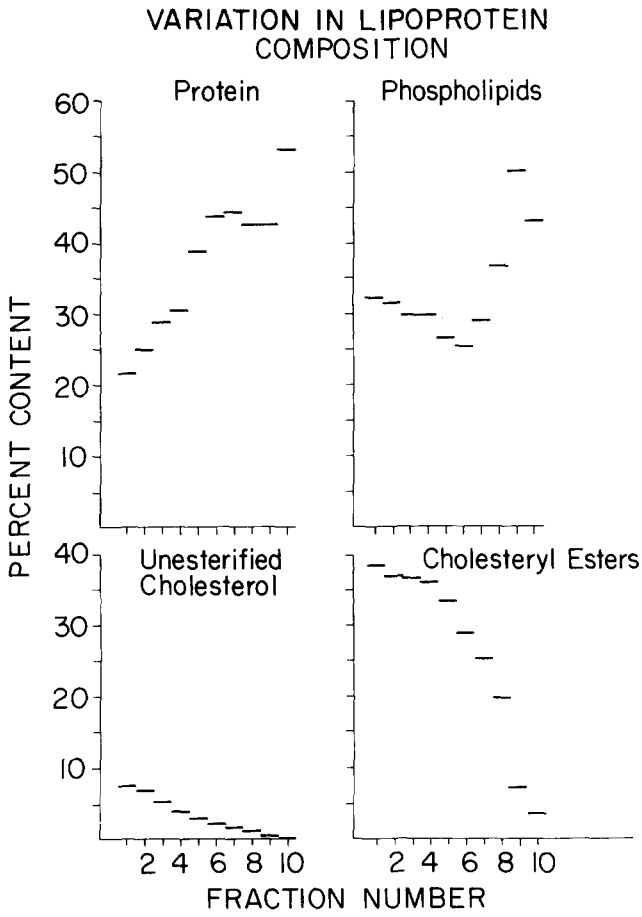

Fig. 1. Bar graph illustrating the variation in the percent composition among the ten ultracentrifugal fractions.

(0.005%). The densities of salt solutions, prepared according to Lindgren (7), and recovered lipoprotein-containing fractions were measured at 20°C to an accuracy of ±0.0002 g/ml using a DMA 02D Mettler/Paar Densitometer (Graz, Austria). Sequential isolation of lipoproteins (ρ < 1.006 g/ml; ρ 1.006–1.050 g/ml; ρ 1.050–1.21 g/ml) using the 40.3 rotor was performed at 39,000 rpm and 16°C in a Beckman L5-65 ultracentrifuge. Lipoproteins were recovered in 2-ml volumes at the end of the first two ultracentrifugal runs and in 1-ml volumes at the conclusion of the third ultracentrifugal run. Separate 2-ml aliquots of the 1.050–1.21 g/ml fraction, concentrated 6-fold over serum levels, were transferred to 1.4 × 8.0 cm cellulose nitrate tubes. The bottom layer was gently overlayed with 6 ml of a 2:1 mixture of the 1.050–1.21 g/ml fraction and a 0.196 molal NaCl solution. This intermediate zone had a density of 1.14 g/ml. The tubes were filled with 4.5 ml of a 0.196 molal NaCl, 1.000 molal NaBr solution (ρ = 1.050 g/ml). Bovine alpha lipoproteins are enriched in carotenoids, primarily beta carotene. As a result, solutions containing a high concentration of these lipoproteins vary in color from bright yellow to dark orange. Thus, in forming the gradient, one is able to monitor visibly the stability of each layer. In spite of the mass of lipoproteins in the two bottom layers, no streaming was observed. Following 40 hr of centrifugation at 37,000 rpm and 16°C in a Beckman SW 41 rotor, ten 1.2-ml fractions were collected from the top of six tubes using an ISCO Density Gradient Fractionator (Lincoln, NE). At the conclusion of the run and during the collection of fractions, the absence of schlieren patterns and convective streamlines indicated that the lipoproteins were spread into stable zones within the gradient that had formed.

Chemical analysis

Using the Autoanalyzer II (Technicon, Tarrytown, NY), cholesterol and triglycerides were measured in isopropyl extracts of sera and of the 1.05–1.21 g/ml density fractions (8). Detailed compositional studies were carried out on the ten fractions, Total and free cholesterol were determined enzymatically (9). Triglycerides were measured in isopropyl extracts of these samples using the technicon Autoanalyzer II (8). Phosphorus analyses were performed as described by Turner and Rouser (10). Protein concentrations were determined by a modified Lowry procedure employing 1% SDS in a $NaCO_3$ buffer (11). Polyacrylamide gel electrophoresis of the apoproteins was performed in 0.1% SDS using a modification (12) of the procedure of Laemmli (13). Gels were scanned with an MK-IIIC Joyce-Lobel Densitometer (Gateshead-On-Tyme, United Kingdom).

Electrophoretic analysis

After being dialyzed against a 0.196 molal NaCl solution at 4°C, the fractions were studied by electrophoresis on agarose (14) and paper (15). Agarose slides were obtained from Bio-Rad (Richmond, CA). Lipoprotein fractions were also analyzed electrophoretically on 5.5% native acrylamide gels, prepared as described by Ornstein and Davis (16).

Hydrodynamic studies

Analytical ultracentrifugation was performed according to the technique of Schumaker et al. (17) using a Beckman Model E ultracentrifuge equipped with a scanner optical system set at a wavelength of 280 nm. Sedimentation and flotation coefficients were measured at 52,000 rpm in NaCl solutions of ρ = 1.011 g/ml, and NaBr–NaCl solutions of ρ = 1.052 and 1.200 g/ml. Intensity fluctuation spectroscopy as described by Kunitake et al. (18) was used to obtain diffusion coefficients in NaCl solutions having densities of 1.010 g/ml.

TABLE 1. Concentrations[a] (mg/ml) and percent content of components in the ten ultracentrifugal fractions

	Fraction									
	1	2	3	4	5	6	7	8	9	10
Jersey										
PRO[b]	2.25 ± 0.04 (22.3)	5.03 ± 0.06 (25.1)	10.92 ± 0.35 (29.3)	12.32 ± 0.72 (29.9)	8.62 ± 0.80 (36.1)	5.83 ± 0.07 (41.2)	3.53 ± 0.05 (43.7)	1.58 ± 0.03 (42.0) n = 2	1.03 ± 0.01 (45.4) n = 2	1.69 ± 0.01 (54.2) n = 2
PL[b]	3.32 ± 0.10 (32.9)	6.16 ± 0.06 (30.7)	10.82 ± 0.20 (29.0)	12.17 ± 0.20 (29.6)	6.61 ± 0.03 (27.7)	4.06 ± 0.07 (28.7)	2.46 ± 0.10 (30.5)	1.40 ± 0.03 (37.2)	1.21 ± 0.04 (53.3)	1.39 ± 0.03 (44.6)
CE[b]	3.71 ± 0.13 (36.7)	7.60 ± 0.24 (37.9)	13.88 ± 0.39 (37.2)	15.15 ± 0.07 (36.8)	7.98 ± 0.25 (33.5)	3.98 ± 0.01 (28.1)	1.93 ± 0.04 (23.9)	0.73 ± 0.00 (19.4)	0.03 ± 0.00 (1.3)	0.03 ± 0.01 (1.0)
UC	0.82 ± 0.00 (8.1)	1.27 ± 0.01 (6.3)	1.71 ± 0.04 (4.6)	1.52 ± 0.02 (3.7)	0.64 ± 0.00 (2.7)	0.29 ± 0.01 (2.1)	0.15 ± 0.01 (1.9)	0.05 ± 0.00 (1.3)	—	0.01 ± 0.01 —
Holstein										
PRO	0.65 ± 0.02 (21.2) n = 2	3.78 ± 0.15 (24.4)	9.80 ± 0.21 (27.9)	13.60 ± 0.96 (30.7)	11.22 ± 0.60 (38.7)	7.23 ± 0.18 (45.9)	3.47 ± 0.37 (44.6)	1.33 ± 0.01 (42.8) n = 2	0.85 ± 0.02 (39.5) n = 2	1.42 ± 0.15 (52.0) n = 2
PL	0.96 ± 0.01 (31.4)	4.98 ± 0.03 (32.1)	10.63 ± 0.12 (30.3)	13.17 ± 0.10 (29.7)	7.30 ± 0.08 (25.2)	3.45 ± 0.13 (21.9)	2.11 ± 0.00 (27.1)	1.11 ± 0.03 (35.7)	1.00 ± 0.03 (46.5)	1.13 ± 0.02 (41.4)
CE	1.24 ± 0.02 (40.5)	5.56 ± 0.04 (35.8)	12.68 ± 0.46 (36.1)	15.73 ± 0.39 (35.5)	9.66 ± 0.13 (33.3)	4.72 ± 0.07 (29.9)	2.09 ± 0.02 (26.9)	0.63 ± 0.01 (20.3)	0.28 ± 0.01 (13.0)	0.17 ± 0.02 (6.2)
UC	0.21 ± 0.00 (6.9)	1.19 ± 0.01 (7.7)	2.00 ± 0.06 (5.7)	1.80 ± 0.00 (4.1)	0.82 ± 0.02 (2.8)	0.36 ± 0.02 (2.3)	0.11 ± 0.00 (1.4)	0.04 ± 0.00 (1.3)	0.02 ± 0.01 (1.0)	0.01 ± 0.01 —

[a] Concentrations are given as means of measurements ± standard deviations.

[b] PRO (protein), unless otherwise indicated measurements were done in quadruplicate; PL (phospholipid), measurements done in triplicate; CE (cholesteryl esters), obtained by subtracting value for unesterified cholesterol (UC) from total cholesterol and dividing difference by 0.6. Cholesterol measurements were done in duplicate.

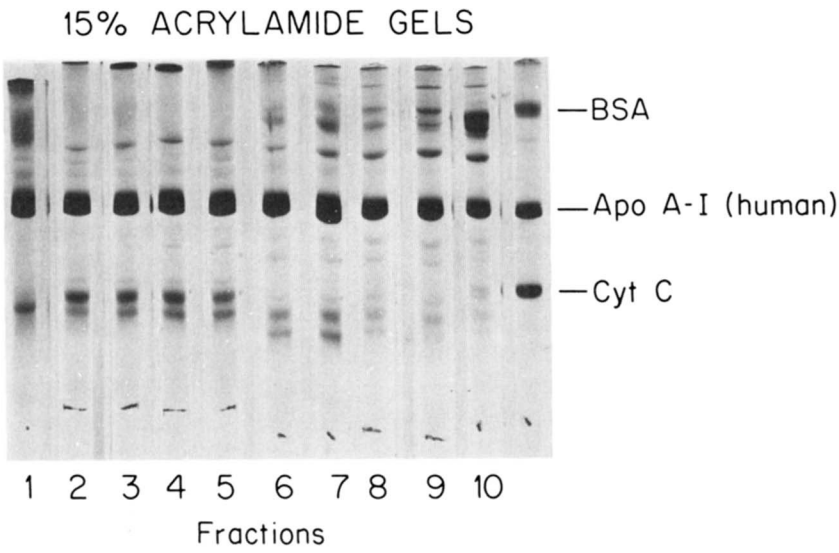

Fig. 2. Reduced apoproteins isolated from fractions 1 to 10. Approximately 50 μg of protein applied to SDS-polyacrylamide gels.

RESULTS

Whole serum data

The total serum cholesterol varied between 100 and 200 mg/dl among the cows in this study. Of this total, 70% to 90% of the cholesterol could be isolated within the density interval between 1.05 to 1.21 g/ml. In contrast, serum triglyceride concentrations were low (10–20 mg/dl), and the 1.05–1.21 g/ml fractions were not enriched in this lipid.

Composition of individual fractions

Compositional variations among the ten fractions are illustrated in **Fig. 1.** The percent content of protein increased from 22% (Fraction 1) to about 43% (Fraction 6 to 9). Both the phospholipid and cholesteryl ester contents dropped steadily from fractions 1 to 6, after which there was a sharp rise in phospholipid content along with a corresponding drop in cholesteryl ester content. A striking decrease was observed in the percentage of unesterified cholesterol across the gradient, resulting in an increase in the molar ratio of esterified to unesterified cholesterol from 3 at the top of the gradient to 8 at the bottom. A similar change in this ratio has been reported for fractions of human HDL (19, 20). Concentrations of individual components are given in **Table 1.** The content of triglycerides was less than 1% of the total mass of each fraction. Fifty percent of the total lipoprotein mass in the gradient was recovered in fractions 3 and 4. Although complete compositional analyses of each fraction were done on only two of the cows, cholesterol and protein determinations indicated that the lipoproteins were distributed similarly in the five other cows studied.

A photograph of SDS-gels is shown in **Fig. 2.** Densitometric scans of these gels indicated that apoA-I was the major protein of fractions 1 through 10. A molecular weight determination based on the average mobilities of the ten gel analyses yielded a mean value of 28,400 ± 970. The other striking features were the presence of proteins with molecular weights of less than 12,000 among the density fractions (primarily 1 through 5) and higher molecular weight proteins particularly among the higher density fractions (6 through 10). Five low molecular weight proteins have previously been isolated from bovine HDL and two of these were found to activate milk lipoprotein lipase (21). Presumably, the low molecular weight apoproteins seen in the gels in Fig. 2 are C apoproteins. A discernible band, corresponding to a 46,000 dalton polypeptide, was observed in all ten gels. The intensity of this band increased as the density of the fraction increased. This protein might represent a bovine form of apoA-IV. Still higher molecular weight polypeptides were in fractions 6 through 10. One of these, corresponding to BSA, was particularly enriched in fractions 9 and 10. Whether or not the other high molecular weight bands correspond to serum proteins or to HDL apoproteins is not known at this time.

Electrophoretic data

The electrophoretic distributions of the various fractions on agarose slides are shown in **Fig. 3.** Clearly discernible alpha bands were seen in fractions 1 through 8. A very faint band was observed in 9 and none was

observed in fraction 10, undoubtedly because the content of esterified neutral lipid, required for optimal lipid staining (15), was low in this fraction.

When the electrophoretograms for bovine whole sera (WP) are compared with that for human plasma (control), a minor band migrating with pre-beta mobility can be seen. This minor band was also observed in fractions 1, 2, and 3 but its intensity decreased as the density of the fraction increased. Presumably this band consisted of beta lipoproteins with more negative charge than their human counterparts. Other mammals have been reported to have beta lipoproteins that electrophorese in a corresponding fashion (22). Similar data (not shown) were obtained on paper, but agarose proved to be the more sensitive media. On paper a minor band was observed only for fraction 1, but not for 2 and 3, even though the paper strips contained more than five times the sample volume applied to the agarose gels.

The distribution of intact lipoproteins of each fraction when electrophoresed on native gels is shown in **Fig. 4.** The mobility of the broad major band was seen to increase as the density of the fractions increased. The exact nature of the two sharp minor bands, seen migrating ahead of the major band in fractions 1 and 5, is unknown at this time. These leading minor bands had identical mobilities in each of the top six fractions and have been seen consistently when these less dense bovine fractions have been analyzed in this manner. Aligning the gels and determining the extent of overlap between the major lipoprotein band in a given fraction and that in a less dense adjacent fraction, the percentage of overlap varied from 70% for fractions 1 and 2 and for fractions 5 with 4 to 12% for fractions 3 and 4. In fraction 6, in which the major band had a higher mobility than the two sharp minor bands, there was no overlap between the major band in fractions 5 and 6. Moreover, among the top six

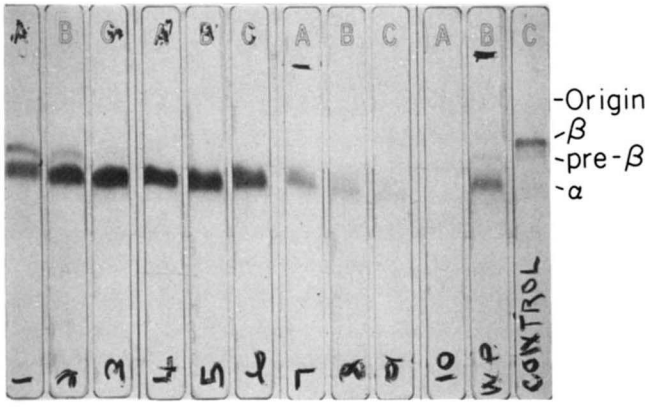

Fig. 3. Agarose electrophoretograms of bovine lipoproteins in fractions 1 to 10. Bovine whole sera and human plasma are designated WP and control, respectively.

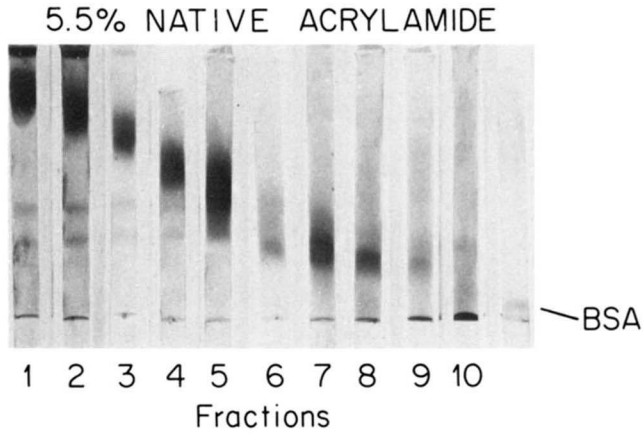

Fig. 4. Native 5.5% polyacrylamide electrophoretograms of bovine lipoproteins fractions 1 to 10. Gels were stained for protein with Coomassie dye.

fractions which contain 90% of the lipoproteins in the gradient, overlap only occurred between the major bands of adjacent fractions.

Sedimentation velocity studies

Sedimentation velocity runs were carried out in dilute NaCl solutions (ρ = 1.010 g/ml). The calculated $s_{20,w}$ values for fractions 1 through 7 obtained from three different cows are listed in **Table 2.** Little variation in sedimentation rate was noted in the values not only for a given fraction among animals but also for different fractions of an individual animal. Evidently compensatory density and size changes were occurring, since clear cut differences among fractions were seen when additional measurements were made in NaBr–NaCl solutions of ρ = 1.052 g/ml and ρ = 1.200 g/ml. The resulting data obtained at these higher densities for the major Guernsey HDL fractions are also shown in Table 2. In both sedimentation and flotation studies, a minor component was observed in fractions 1 and 2. It was more prominent in fraction 1.

Density calculations

From both the compositional data presented in Table 1 and sedimentation velocity data presented in Table 2, the lipoprotein densities were calculated for fractions 1 through 7. The densities were calculated for the Guernsey data by plotting ηs vs ρ to obtain the density intercept corresponding to a zero sedimentation rate. The results of both calculations listed in **Table 3** are in close agreement with the measured solution densities for each fraction.[3] The analyses indicate that lipoprotein fractionation

[3] Published values for partial specific volumes for lipid (23) and protein (24) components were used to calculate density from the compositional data in Table 1.

TABLE 2. Sedimentation rates of the fractionated bovine alpha lipoproteins

	Fraction						
	1	2	3	4	5	6	7
$S_{20,w}$[a]							
Jersey	4.66	4.88	5.11	5.29	5.15	5.13	5.11
Holstein	5.46	5.44	5.78	5.55	5.32	5.20	5.31
Guernsey	5.67	5.92	5.99	5.83	5.75	5.48	5.51
$S_{20,1.0518}$[a]							
Guernsey			1.67	2.08	2.63	3.49	3.64
$F_{20,1.2004}$[b]							
Guernsey	14.57	11.72	8.59	6.72	5.45	3.19	2.47

[a] $S_{20,w}$ and $S_{20,1.0518}$, sedimentation coefficients expressed in Svedbergs and corrected to reference solvents having at 20°C the viscosities and densities of water and a NaBr solution (d = 1.0518 g/ml), respectively.

[b] $F_{20,1.2004}$, negative sedimentation coefficient expressed in Svedbergs and corrected to a reference solvent having at 20°C the viscosity and density of a NaBr solution (d = 1.2004 g/ml).

was achieved in an isopycnic gradient according to density.

Intensity fluctuation spectroscopy

Diffusion coefficients for fractions 3, 4, and 5 were determined from intensity fluctuation spectroscopy measurements (**Table 4**). Knowledge of the hydrated densities listed in Table 3, and $S_{20,w}$ and $D_{20,w}$ values enabled calculation of molecular weights, frictional ratios, and the Einstein-Stokes radii. These results for fractions 3, 4, and 5 appear in Table 4.

DISCUSSION

Several sedimentation studies (25, 26) of bovine high density lipoproteins are consistent with a homogeneous population of particles. However, flotation analyses (6, 26–28) as well as gel filtration (29) and electron microscopic studies (6, 26–28) have shown that particles vary both in size and density. By separating the alpha lipoproteins of the lactating cow into ten fractions, we have been able to obtain physiocochemical data on the major fractions. Our results indicate that a considerable physical and chemical heterogeneity exists among these macromolecules.

Lipoproteins in all but fraction 10 exhibited alpha mobility on agarose gels. A minor band with the mobility of human pre-beta lipoproteins was present in the electrophoretograms of the top three fractions from the gradient. Bovine low density lipoproteins have previously been shown to be electrophoretically heterogeneous (1, 6, 29–33). The minor bands observed in the agarose gel most probably consist of beta lipoproteins more negatively charged than their human counterparts. However, based on their density and electrophoretic mobility, they also could correspond to that class of human lipoproteins known as Lp(a) (34). Examination of the tops of the SDS-acrylamide gels for fractions 1 through 4 (See Fig. 1) revealed a band, presumably the large molecular weight apoprotein B, which we have assumed to be associated principally with the lipoproteins exhibiting pre-beta mobility.

The apoprotein distribution within the SDS-polyacrylamide gels revealed minor bands having molecular weights greater and less than apoA-I. Fractions 1 through 5 were particularly enriched in apoproteins having molecular weights less than 12,000. On the other hand, SDS gels of fractions 6 through 10 contained minor bands with molecular weights between 40,000 and 70,000. The presence of what appears to be C-apoproteins among the less dense fractions of bovine alpha lipoproteins is in agreement with results published by Shore and Shore (35), who found that the distribution of the human C-apoproteins varied among different HDL subclasses. These differences in the distribution of the minor apoproteins possibly are related to metabolic origins or functions of these fractions of alpha lipoproteins in the lactating cow. With the onset of lactation, there is a marked increase in the concentration of the large alpha lipoproteins (2). It has been proposed that increased plasma levels of large alpha lipoproteins might be a metabolic response to make more surface available for C-apoproteins (2). Such an increase in the C-apo-

TABLE 3. Comparison of calculated lipoprotein densities with measured fraction densities

	Fraction						
	1	2	3	4	5	6	7
Isopycnic solution density (g/ml)	1.051	1.058	1.069	1.083	1.100	1.117	1.135
Density based on composition							
Jersey	1.058	1.065	1.076	1.079	1.099	1.119	1.130
Holstein	1.052	1.065	1.074	1.082	1.106	1.130	1.130
Density based on sedimentation rate data							
Guernsey	1.046[a]	1.057[a]	1.072	1.082	1.093	1.119	1.131

[a] In the case of fractions 1 and 2, analyses were done only in solutions of density 1.01 and 1.20 g/ml.

protein reservoir during lactation presumably would act to enhance the activity of lipoprotein lipase of the mammary gland, thereby enabling fatty acids released from circulating triglycerides to be incorporated into milk fat. Alternatively, or in addition, this increase could reflect the conversion of surface components derived from catabolized triglyceride carriers into these large alpha lipoproteins (2). The 46,000-molecular weight apoprotein, if it corresponds to a bovine form of apoA-IV, might also reflect the conversion of triglyceride-rich lipoproteins into the alpha lipoproteins in fractions 7 through 10.

In all of the bovine fractions the major apoprotein was found to correspond to apoA-I. In other studies, we have found this to be true for bovine alpha lipoproteins with densities as low as 1.03 g/ml. Similar low density alpha lipoproteins have also been reported in the plasma of other mammals (6, 36–39). In this regard, these bovine alpha LDL are not unlike large human alpha lipoproteins (6), which also are particularly enriched in apoA-I and contain very little apoA-II (40, 41). On the other hand, they differ from the alpha lipoproteins that can be isolated from the plasma of mammals fed diets high in cholesterol (42). The density distribution of these lipoproteins, induced by high cholesterol diets, also extends into the low density class, but their protein moiety is particularly enriched in the E apoproteins (42).

Our data on the lipid composition of the major fractions are in good agreement with most previous studies on bovine HDL (1, 27, 28, 43–45). Circulating triglyceride levels in ruminants are typically lower than in human plasma (6, 27, 28, 43–50). Over 80% of bovine triglycerides are transported by lipoproteins with density less than 1.020 g/ml (44). On the other hand, high density lipoproteins, which comprise the major lipoprotein class in these mammals, have a very low triglyceride content (5% or less) (1, 27, 28,43–45). Bovine HDL, having higher triglyceride contents of 20% (26) and 11% (25) have been reported in two separate studies; however, the total plasma triglyceride levels were not measured. Considering both the low triglyceride levels and the high HDL levels usually measured in bovine plasma, it is difficult to explain these two reports of a relatively high content of triglycerides in bovine HDL. Because, in these two studies, the published triglyceride values were determined by a difference calculation and were not measured directly, an error inherent in such determinations may have given rise to the discrepancies.

Our combined hydrodynamic data derived from sedimentation and flotation velocity runs, hydrated density calculations, diffusion measurements, and molecular weight calculations, clearly demonstrated the heterogeneous nature of the alpha lipoproteins in the lactating cow. Three of the major subfractions 2, 3, and 4, had flotation rates greater than HDL obtained from normal human subjects (19). Similar observations have been made in previous flotation rate studies of bovine HDL (2, 6, 26–28). Lipoproteins, exhibiting a broad range in flotation rates and distributed asymmetrically about a peak with an $F_{1.20}$ value of 6 to 8, were reported for the HDL in the sera of both a lactating cow (2, 6) and a steer (27, 28). In a study of heifer HDL (26), Jonas found a single symmetrical, but broadly distributed peak. In a less dense 0.1 M phosphate solution, the same material reportedly sedimented as a very sharp, homogeneous peak. Similar observations have been made in other sedimentation analyses of bovine HDL (25, 48). This apparent homogeneity of bovine HDL in sedimentation velocity studies is consistent with our data in Table 3. We noted little variation in the $S_{20,w}$ values among the major fractions. This appears to be due to a compensatory decrease in particle density as molecular weight increases, resulting in a relatively constant value for the term $M^{2/3}(1 - \phi\rho_{20,w})\phi,^{-1/3}$ which determines the value of the sedimentation coefficient.

TABLE 4. Molecular weight, frictional ratio, and Stokes radius derived from hydrodynamic measurements

	Fraction		
	3	4	5
Holstein			
$D_{20,w}$	2.86	3.53	3.56
$M(\times 10^{-5})$	7.44	4.89	3.94
f/f_0	1.15	1.07	1.15
R_s (nm)	6.5	5.7	5.2
Guernsey			
$D_{20,w}$	3.39	4.16	3.74
$M(\times 10^{-5})$	6.50	4.36	4.05
f/f_0	1.02	0.94	1.08
R_s (nm)	6.2	5.5	5.3

Symbols: $D_{20,w}$, diffusion coefficient expressed in Ficks and corrected to a reference solvent having the viscosity of water at 20°C; M, molecular weight; f/f_0, frictional ratio; R_s, Stokes radius.

Lipoprotein densities of individual fractions calculated from velocity measurements made in three different solutions agreed well with the solution densities of the fractions, as well as the densities calculated from the composition of each fraction. Three other density values have been reported for bovine HDL. Although heifer HDL was found to have a higher flotation rate than human HDL, Jonas (26) calculated a density of 1.10 g/ml based on concentration and pycnometry measurements. Utilizing velocity measurements, Stead and Welch (51) reported a value of 1.071 g/ml for lactating cow HDL and Dryden et al. (48) reported a value of 1.077 g/ml for steer HDL. These two lower values are in close agreement with our values for fraction 3.

Intensity fluctuation spectroscopy measurements enabled the determination of diffusion coefficients for fractions 3, 4, and 5. These values together with parameters listed in Tables 2 and 3 were used to calculate both the molecular weight, frictional ratios, and Einstein-Stokes

radii. Stead and Welch (51) found a molecular weight value of 580,000 for bovine HDL. Swaney (52) reported data which could be used to calculate an HDL molecular weight in excellent agreement with this value of Stead and Welch (51). Utilizing techniques to cross-link the apoproteins, Swaney estimated the number of apoA-I on the surface of HDL isolated from several types of plasma. His data for bovine HDL indicated that as many as six apoA-I could be present on the surface. Taking the average value of protein content for our two major fractions 3 and 4 (vis. 29.5%), a value of 570,000 is obtained, a result intermediate among our calculated values for fractions 3 and 4.

Isolation of subfractions of human HDL has been accomplished in gradients generated in angle head (19), swinging bucket (41), and zonal rotors (20). In each of these studies, the densities of the various fractions were determined after the gradients had reached an equilibrium state, requiring 48 hr or more of centrifugation. In our study, isopycnic separation of bovine HDL was achieved after just 40 hr of ultracentrifugation. Recently, in studies of whole plasma, we have eliminated the three preceding spins with the 40.3 rotor and have successfully fractionated bovine, mouse, human, and non-human primate alpha lipoproteins in a single spin with the swinging bucket rotor. The same three layer gradient described in the Method Section was employed, except the bottom 2 ml consisted of a salt solution–plasma (1.1 ml) mixture, adjusted to a density of 1.21 g/ml and the 6-ml intermediate zone was a salt solution of density 1.14 g/ml. Techniques reported in this study have proved to be sufficiently sensitive to characterize the major fractions obtained in this manner from plasma. Based on these findings, we feel that our isopycnic gradient can be utilized to fractionate and characterize the major alpha lipoproteins present in a wide variety of mammalian plasma. These studies will be described in a future publication.

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