



**Plasma lipoproteins and apolipoproteins, in the preruminant calf, Bos spp: Density distribution, physicochemical properties, and the in vivo evaluation of the contribution of the liver to lipoprotein homeostasis**

Dominique Bauchart, Denys Durand, P. Michel Laplaud, Patricia Forgez, Sylvie Goulinet, M. John Chapman

► **To cite this version:**

Dominique Bauchart, Denys Durand, P. Michel Laplaud, Patricia Forgez, Sylvie Goulinet, et al.. Plasma lipoproteins and apolipoproteins, in the preruminant calf, Bos spp: Density distribution, physicochemical properties, and the in vivo evaluation of the contribution of the liver to lipoprotein homeostasis. Journal of Lipid Research, 1989, 30 (10), pp.1499-1514. hal-02728216

**HAL Id: hal-02728216**

**<https://hal.inrae.fr/hal-02728216>**

Submitted on 2 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Plasma lipoproteins and apolipoproteins in the preruminant calf, *Bos* spp: density distribution, physicochemical properties, and the in vivo evaluation of the contribution of the liver to lipoprotein homeostasis

Dominique Bauchart,<sup>1,\*</sup> Denys Durand,<sup>\*</sup> P. Michel Laplaud,<sup>†,\*\*</sup> Patricia Forgez,<sup>†</sup> Sylvie Goulinet,<sup>†</sup> and M. John Chapman<sup>†</sup>

Laboratoire d'Etude du Metabolisme Energétique,<sup>\*</sup> INRA, Theix, 63122 Ceyrat, France; INSERM U. 321, Unité de Recherches sur les Lipoprotéines et Athérogénèse,<sup>†</sup> Pavillon Benjamin Delessert, Hôpital de la Pitié, 83 boulevard de l'Hôpital, 75651 Paris Cedex 13, France; and Laboratoire de Biochimie Médicale,<sup>\*\*</sup> Faculté de Médecine et de Pharmacie, 2 rue du Docteur Marcland, 87025 Limoges Cedex, France

**Abstract** The in vivo role of the liver in lipoprotein homeostasis in the preruminant calf, a functional monogastric, has been evaluated. To this end, the hydrodynamic and physicochemical properties, density distribution, apolipoprotein content, and flow rates of the various lipoprotein particle species were determined in the hepatic afferent (portal vein and hepatic artery) and efferent (hepatic vein) vessels in fasting, 3-week-old male preruminant calves. Plasma lipoprotein profiles were established by physicochemical analyses of a series of subfractions isolated by isopycnic density gradient ultracentrifugation. Triglyceride-rich very low density lipoproteins (VLDL) ( $d < 1.018$  g/ml) were minor plasma constituents ( $\sim 1\%$  or less of total  $d < 1.180$  g/ml lipoproteins). The major apolipoproteins of VLDL were apoB-like species, while the complement of minor components included bovine apoA-I and apoC-like peptides. Particles with diameters (193–207 Å) typical of low density lipoproteins (LDL) were present over the density interval 1.026–1.076 g/ml; however, only LDL of  $d$  1.026–1.046 g/ml were present as a unique and homogeneous size subspecies, containing the two apoB-like species as major protein components in addition to elevated cholesteryl ester contents. LDL represented  $\sim 10\%$  of total  $d < 1.180$  g/ml lipoproteins in fasting plasma from all three hepatic vessels. Overlap in the density distribution of particles with the diameters of LDL and of high density lipoproteins (HDL) occurred in the density range from 1.046 to 1.076 g/ml; these HDL particles were 130–150 Å in diameter. HDL were the major plasma particles ( $\sim 90\%$  of total  $d < 1.180$  g/ml substances) and presented as two distinct populations which we have termed light (HDL<sub>L</sub>) and heavy (HDL<sub>H</sub>) HDL. Light HDL ( $d$  1.060–1.091 g/ml) ranged in size from 120 to 140 Å, and were distinguished by their high cholesteryl ester (29–33%) and low triglyceride (1–3%) contents; apoA-I was the principal apolipoprotein. Small amounts of apolipoproteins with  $M_r < 60,000$ , including apoC-like peptides, were also present. Heavy HDL ( $d$  1.091–1.180 g/ml) accounted for almost half (47%) of total calf HDL, and like HDL<sub>L</sub>, were also enriched in cholesteryl ester and apoA-I; they ranged in size from 93 to 120 Å. The protein moiety of

HDL<sub>H</sub> was distinct in its possession of an apoA-IV-like protein ( $M_r$  42,000). Blood flow rates were determined by electromagnetic flowmetry, thereby permitting determination of net lipoprotein balance across the liver. VLDL were efficiently removed during passage through the liver (net uptake 1.06 mg/min per kg body weight). LDL showed minor concentration differences across the liver; indeed, the sum of hepatic uptake, interconversion, and secretion processes showed only a slight negative balance ( $-0.44 \pm 1.06$  mg/min per kg body weight) although marked variation was seen between animals. Measurements of hepatic HDL flux showed a net production of light HDL ( $+5.92 \pm 5.53$  mg/min per kg body weight) and a net uptake of heavy HDL ( $-11.34 \pm 4.47$  mg/min per kg body weight).

These findings are indicative of intense metabolism of HDL subpopulations during passage through the liver in the calf. Finally, the three- to sixfold higher levels of VLDL in the portal vein as compared to hepatic artery and hepatic vein suggest a direct secretion of intestinal VLDL into this vessel.

—Bauchart, D., D. Durand, P. M. Laplaud, P. Forgez, S. Goulinet, and M. J. Chapman. Plasma lipoproteins and apolipoproteins in the preruminant calf, *Bos* spp: density distribution, physicochemical properties, and the in vivo evaluation of the contribution of the liver to lipoprotein homeostasis. *J. Lipid Res.* 1989. 30: 1499–1514.

**Supplementary key words** density gradient ultracentrifugation • analytical ultracentrifugation • gradient gel electrophoresis • hepatic vessel cannulation • electromagnetic flowmetry

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins, with densities as defined; apo, apolipoprotein; LCAT, lecithin:cholesterol acyltransferase; EDTA, ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulfate; BW, body weight; PV, portal vein; HA, hepatic artery; HV, hepatic vein.

<sup>1</sup>To whom correspondence should be addressed.

To date, studies of the lipid transport system in cattle (*Bos* spp) have been primarily focused on qualitative and quantitative aspects of the plasma lipoprotein profile, on the physical and chemical characteristics of the major lipoprotein particles and their apolipoproteins, and on the utilization of fatty acids by mammary and muscular tissues (1–5). With the exception of the fetal calf (3), this system is typically dominated by high density lipoproteins (HDL) and apolipoprotein A-I (2–13), but may, under certain conditions, exhibit considerable variation as a function of physiological, developmental, or nutritional state, and particularly in relation to the gestation-lactation cycle (4, 10, 14–16). Such variation is frequently accompanied by marked complexity in the structure, distribution, and metabolism of certain classes of lipoproteins, a case in point being the complex spectrum of low density particles distributed over the density range 1.006–1.063 g/ml in lactating cows (4, 15, 17, 18).

The digestive physiology of bovine species, and particularly the possession of a rumen, is a key feature in determining fundamental aspects of the absorption, transport, storage, and utilization of lipids of dietary origin in these animals (1, 19, 20). In adult cattle then, fermentation of dietary components in the rumen is associated with extensive hydrolysis of lipids and biohydrogenation of the bulk of fatty acids by the combined action of bacteria and protozoa (1, 20). As a consequence, the fatty acids that are absorbed from the small intestine are primarily saturated in nature and directly influence the physicochemical properties of the lipoprotein particles in which they are transported as well as the lipid composition of tissues and milk (1, 4, 15, 19).

The digestive system of the postnatal calf is, however, distinct from that of the adult bovine. Thus, from birth to the age of 1–2 months, the reticulorumen of the calf is poorly developed. Milk, the sole dietary constituent, induces a closing reflex of the esophageal groove, thereby allowing direct passage of digesta to the forestomachs. This specific digestive feature is characterized by the absence of fermentation processes, and no microbial modification of the chemical structure of dietary lipids occurs. On this basis, the calf, a so-called preruminant, may be considered a functional monogastric.

The available data on the physicochemical characteristics of bovine plasma lipoproteins and of their apolipoproteins have been summarized in several recent reviews (2, 4, 5). Despite extensive investigations at the plasma level, however, the role of the liver in lipoprotein metabolism remains ill-defined, although hepatic disorders may occur frequently in these species; examples are the steatosis of early lactation in high milk-producing dairy cows (21), and the lipid infiltration and inflammatory lesions of the liver associated with the stunting of growth in preruminant calves fed a high-fat milk diet (22, 23).

In line with our ongoing studies of the mechanisms of

lipid absorption, transport, and utilization in the preruminant calf (22–26), we developed an integrated approach. This approach involved the simultaneous determination of blood flow rates in the afferent (hepatic artery and portal vein) and efferent hepatic blood vessels (hepatic vein), together with the qualitative and quantitative characteristics of plasma lipoprotein and apolipoprotein profile in each of these vessels. In this way, we have been able to evaluate, for the first time, in vivo lipoprotein fluxes across the liver in *Bos* spp.

## MATERIALS AND METHODS

### Animals and diets

In our experiments, four crossbred Friesian-Holstein male calves were used. These animals were provided by the Laboratory of Research on Lactation (INRA, Theix, France) and were 5 to 7 days of age. They were housed individually in wooden stalls on a litter of wood shavings in an air-conditioned room (average temperature, 20°C; humidity, 80 %).

Liquid milk replacer was bucket-fed in two equal meals per day (0900 h and 1600 h), and contained 16 % dry matter (gross energy, 5,182 kcal/g) which was composed of 68 % spray-dried skim milk powder (22.8 weight % protein), 23 % tallow, 6.8 % corn starch, and 2.2 % vitamin and mineral mixture (Roquette Frères, Lestrem, France). The total lipid and fatty acid content of milk powder amounted to 24.1 % and 22.6 % of the dry matter, respectively; the principal fatty acids were palmitic (27.6 % of total), stearic (18.6 %), oleic (35.1 %), and linoleic (3.5 %) acids.

At the time of operation at 10 days of age (see below), the calves weighed  $45.1 \pm 3.0$  kg. At blood sampling (18 to 21 days old), they had a growth rate of  $610 \pm 115$  g/day for a dry matter intake amounting to 60 g/kg metabolic body weight ( $\text{wt}^{0.75}$ ).

### Blood samples

Four calves were fasted overnight for approximately 17 h. Thirty ml of blood was simultaneously collected from each of the three catheterized vessels (see below) on Na<sub>2</sub>-EDTA (final concentration 1 mM). Plasma was then separated by centrifugation at 3500 rpm for 15 min and antibacterial agents were added (sodium merthiolate and sodium azide at final concentrations of 0.001% and 0.01% (w/v), respectively). Plasma was maintained at 4°C until lipoprotein fractionation was initiated and typically within 48 h of its isolation (see lipoprotein isolation below).

### Surgical techniques and estimation of blood flow

Determination of the rates of blood flow throughout the liver was carried out in calves fitted with both chronic

catheters and electromagnetic blood flow probes in their hepatic vessels (portal and hepatic veins, hepatic artery).

Anesthesia was initially induced with 4.5% halothane in oxygen (ICI Pharma, France) using a face mask and extended with 2% halothane in oxygen during the overall operation. The portal vein catheter (polyvinyl 1.5 mm ID  $\times$  2.5 mm OD; Bruneau, Boulogne-Billancourt, France) was introduced into the main mesenteric vein until it lay in juxtaposition to the porta hepatis. The arterial catheter (0.8 mm ID  $\times$  1.2 mm OD) was introduced into a mesenteric artery (similar to the hepatic artery), and the hepatic vein catheter (1.5 mm ID  $\times$  2.5 mm OD) was introduced into the main hepatic vein via the vena cava as described by Durand et al. (27).

Measurements of blood flow were made by electromagnetic flowmetry (Gould Inc., Statham Instruments Div., Oxnard, CA) using two probes placed around the portal vein (15 mm i.d.) and the hepatic artery (left branch; 3 mm i.d.) as outlined by Durand et al. (27). Flow probes were calibrated in vitro using a gravity flow system prior to implantation of the probes and again after slaughter of the animals. The pulsed field wave form of the flowmeter allowed us to establish a base line reference during the entire period of the experiment without mechanical occlusion of the blood vessels (electronic zero).

The extent of stress to the animals was minimal as judged by their mean increase in body weight, 600 g/day.

### Lipoprotein isolation

Chylomicrons ( $S_f > 400$ ) were first removed from plasma by ultracentrifugal flotation at 20,000 rpm ( $2.25 \times 10^6$  g-min) for 45 min at 15°C using a Beckman SW 41 rotor according to Zilversmit (28).

Lipoproteins were then isolated from chylomicron-free plasma by ultracentrifugation in a discontinuous density gradient according to the method of Chapman et al. (29) for human serum lipoproteins, with the exception that 2.5 ml of the top saline solution (d 1.006 g/ml) was used instead of 3 ml. Plasma samples were adjusted to a density of 1.210 g/ml with solid KBr (0.325 g/ml). The density of NaCl-KBr solutions for density gradient construction (with densities of 1.006, 1.019, 1.063, and 1.240 g/ml) was measured at 15°C with a precision of  $\pm 0.0001$  g/ml by means of a digital density meter (Model DMA 46, A. Paar KG, Graz, Austria). As for plasma samples, these salt solutions contained sodium merthiolate (0.001% w/v) and sodium azide (0.01%). Gradient construction was carried out at ambient temperature using a Buchler Autodensiflow II (Buchler Instruments, Searle Analytic Inc, Fort Lee, NJ) at a speed of 1 ml/min. Ultracentrifugation was performed in a Beckman SW41 rotor at 40,000 rpm ( $54.4 \times 10^7$  g-min) for 46 h at 15°C in a Sorvall OTD 65B ultracentrifuge, with no braking at the end of the run.

Plasma-containing gradients were divided into 22 successive fractions of 0.4 ml (0.5 ml for fraction 1 containing VLDL) by stepwise aspiration with a micropipette, but excluding the final 3 ml at the bottom of the tube which consisted of 97.3% protein and 2.7% lipid; the latter (3.6% of total plasma lipids) was composed of 21% free cholesterol, 47% triglyceride, and 32% phospholipid. The density of each fraction was determined by reference to the density profile obtained from control gradients containing only salt solutions (Fig. 1). Lipoprotein fractions were dialyzed in Spectra/Por 4 membrane tubing (no. 132697, molecular cut-off 12,000–14,000, Spectrum Medical Industries Inc, Los Angeles, CA) for 3  $\times$  12 h at 4°C against changes of a solution (6 l for 44 fractions) containing 0.05 M NaCl, 0.005 M  $\text{NH}_4\text{HCO}_3$ , 0.04%  $\text{Na}_2\text{-EDTA}$ , 0.01% sodium azide, and 0.001% sodium merthiolate at pH 7.4.

### Analytical ultracentrifugation

Analyses were performed at two different densities, i.e., 1.210 g/ml for the determination of the overall distribution of plasma lipoproteins and for the pattern of HDL, and 1.063 g/ml for precise determination of LDL. The conditions used for the initial isolation of lipoprotein fractions by preparative ultracentrifugation and subsequently for

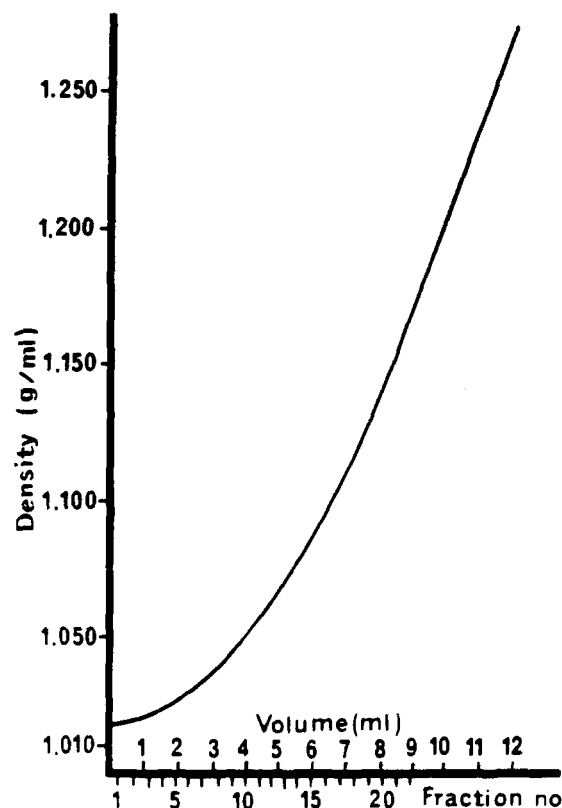


Fig. 1. Density profile determined from control gradients after ultracentrifugation under the conditions described in the Methods section.



analytical ultracentrifugal analysis using Centriscan 75 machine (MSE Crawley, UK) have been described previously (30).

### Gradient gel electrophoresis of native lipoproteins

Evaluation of particle diameter and the potential heterogeneity of lipoprotein size species was performed on fractions isolated from the plasmas of each of the three hepatic vessels by electrophoresis at 15°C on continuous polyacrylamide gradient gel slabs from 4 to 30% (PAA 4/30; Pharmacia, Uppsala, Sweden) (31). Gels were stained with 0.5% Coomassie Brilliant blue (Sigma) and destained in 10% trichloroacetic acid. No attempt was made to quantify the bands detected in these electrophoreses. The Stokes diameters of lipoprotein particles were determined using the Stokes-Einstein equation (31) from a calibration curve constructed from a series of protein markers (Pharmacia) i.e., 71 Å (bovine serum albumin), 81 Å (lactic dehydrogenase), 104 Å (catalase), 122 Å (ferritin), and 170 Å (thyroglobulin).

### Chemical analysis

Protein concentrations were determined in each lipoprotein fraction by the procedure of Lowry et al. (32) using 0.5 M sodium deoxycholate (Fluka, Switzerland) and with bovine serum albumin as the working standard.

Total cholesterol (TC) and free cholesterol (FC) were measured enzymatically using the reagent kit supplied by Merck (CHOD-iodide, Merkotest no. 14350, Darmstadt, West Germany). Cholesteryl ester (CE) content was calculated using the relation  $CE = (TC - FC) \times 1.68$ . Triglyceride was estimated by the enzymatic method of Fosati and Prencipe (33) using Biomérieux reagent kit (PAP 1000, no. 6.123.6; Biomérieux, Charbonnières-les-Bains, France) which determines total glycerol content. The term triglyceride designates the plasma content of triglycerides plus free glycerol, while the content of triglycerides in the various lipoprotein subfractions corresponds specifically to triglycerides since the lipoproteins were dialyzed to remove free glycerol (30).

Quantitation of phospholipid (PL) (as phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin) was determined by the enzymatic method of Trinder as described by Takayama et al. (34). This procedure measures choline liberated after hydrolysis by phospholipase D and involves use of the Biomérieux kit (PAP 150, no. 6.149.1).

### Electrophoretic studies of apoprotein moieties

Aliquots of lipoprotein fractions isolated by gradient density ultracentrifugation were first lyophilized and then delipidated with ethanol-diethyl ether (peroxide-free) 3:1 (v/v) according to Brown, Levy, and Fredrickson (35). Protein moieties were studied in two different electrophoretic systems.

The molecular weights of the constituent apolipoproteins of gradient subfractions 1–22 were first estimated by electrophoresis in an SDS-polyacrylamide gradient gel system constructed according to the procedure of Irving et al. (36), and using a dual vertical slab gel electrophoresis cell (Hoefer Instruments, San Francisco, CA). Slabs with a thickness of 1.5 mm and a length of 28 cm were constructed with a peristaltic pump (Gilson Minipuls II) to give a nonlinear continuous gradient from 3 to 25% acrylamide. More precisely, one volume of light solution (3% acrylamide monomer) was pumped into the heavy solution (25% acrylamide monomer) while one volume was delivered to the slab. After running gel construction, a stacking gel was added in 3% acrylamide and Tris-glycine buffer at pH 8.0 to a height of 3 cm. Samples (100 µg protein of each subfraction) were dissolved in a solution containing 0.5% SDS, 1% β-mercaptoethanol and 10 mM Tris-HCl and incubated for 2 h at 37°C before application to individual sample wells. Electrophoresis was then performed for 20 h at 15–17 mA/slab and at 15°C. On completion of electrophoresis, gels were stained with Coomassie Brilliant Blue R250.

Two calibration curves were constructed from a series of polymerized molecular weight markers (BDH Biochemicals, Poole, UK) ranging in mass from 56,000 to 280,000 and from 14,300 to 71,000 daltons, respectively. Proteins with a molecular weight above 80,000 were compared to a standard curve constructed with the high molecular weight markers, while the size of proteins with molecular weight below this limit was calculated with the second standard curve. In the both cases, the correlation coefficients for these curves were typically greater than 0.99.

Tetramethylurea-soluble apolipoproteins (50 or 100 µg of lyophilized protein) from gradient subfractions 1–22 were electrophoresed in the alkaline-urea polyacrylamide disc gel system of Kane (37) at pH 8.6. Gels (7.5% monomer) were stained with 1% Coomassie Brilliant Blue R250 in 12% trichloroacetic acid and subsequently destained in 10% trichloroacetic acid.

### Calculation of hepatic balance

Lipoprotein hepatic balance was determined from simultaneous blood flow measurements in the portal vein and in the hepatic artery and from the plasma concentrations of each lipoprotein subfraction isolated by gradient density ultracentrifugation from plasmas derived from the portal vein, hepatic artery, and hepatic vein. Plasma flows were corrected for packed-cell volume.

The afferent flow of a given lipoprotein subfraction into the liver represented the sum of the flows in the portal vein and in the hepatic artery, and was calculated by multiplying lipoprotein concentration by the plasma flow as follows.

Hepatic afferent lipoprotein flow (mg/min per kg body wt) =  $C_{PV} \cdot F_{PV} + C_{HA} \cdot F_{HA}$  where  $F_{PV}$  and  $F_{HA}$  are the portal and hepatic arterial plasma flows (ml/min per kg body wt), respectively, and  $C_{PV}$  and  $C_{HA}$  are the portal and hepatic arterial lipoprotein plasma concentrations (mg/ml), respectively.

Hepatic efferent plasma flow was considered as being equal to hepatic afferent plasma flow, which was calculated as:

hepatic efferent lipoprotein flow =  $C_{HV}(F_{PV} + F_{HA})$ , where  $C_{HV}$  is the hepatic venous plasma lipoprotein concentration (mg/ml).

We have therefore assumed that the hepatic balance corresponded to the amount of each lipoprotein subfraction removed (negative balance) or produced (positive balance) by the liver and expressed it as a percentage of the total of these subfractions presented to the liver. Therefore, the hepatic balance could be calculated from the following equation as proposed by Brockman and Bergman (38):

$$\text{Lipoprotein hepatic balance} = \frac{C_{HV}(F_{PV} + F_{HA}) - (C_{PV} \cdot F_{PV} + C_{HA} \cdot F_{HA})}{C_{PV} \cdot F_{PV} + C_{HA} \cdot F_{HA}} \times 100.$$

### Statistical analysis

The nonparametric test of Mann and Whitney (39) was used to estimate the statistical significance of the differences observed between the concentrations of the different lipoprotein classes measured, respectively, at the three sites of blood sampling. We have also evaluated the potential significance of differences in the hepatic balance of individual lipoprotein gradient subfractions using the same nonparametric test.

## RESULTS

### Lipid content of whole plasma

Initially, the concentrations of the major plasma lipids present in the portal and hepatic veins and the hepatic artery of fasting preruminant calves were determined and are shown in **Table 1**. Mean plasma lipid concentrations in all three vessels were characterized by high levels of cholesteryl ester (44.2% of total major lipids) and phospholipids (41.1%), and by low levels of triglycerides (7.4%) and free cholesterol (5.7%). Differences between vessels were noted in the cases of triglyceride and cholesteryl ester, for which plasma levels were the highest in the portal vein and in the hepatic vein (10.6% and 47.6% of total lipids), respectively.

### Characterization of plasma lipoproteins

**Analytical ultracentrifugation.** As a first approach to the characterization of the quantitative aspects of the plasma lipoprotein spectrum in the preruminant calf, analytical studies were undertaken. In this instance, the classical hydrodynamic criteria, developed for estimation of the major lipoprotein classes in man, were applied to the calf.

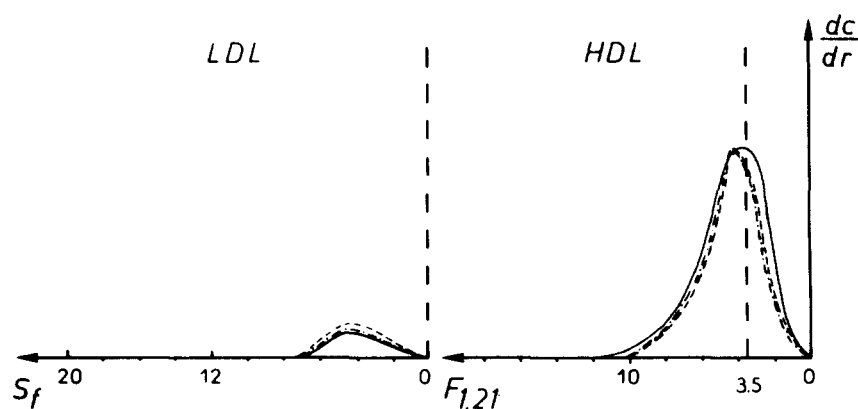
Scans of lipoprotein profiles taken at a density of 1.063 g/ml and corresponding to individual plasma samples from each of four fasting calves (**Fig. 2**) revealed the presence of very low amounts of VLDL; these lipoproteins were almost undetectable in the hepatic artery and hepatic vein. Therefore, precise quantitation of such low amounts of particles was impracticable, as was the accurate determination of the profile of the distribution of lipoproteins with  $S_f$  20–400. IDL were undetectable by analytical ultracentrifugation. The 1.006–1.063 g/ml density class was thus represented primarily by LDL with a mean peak  $S_f$  rate of  $4.6 \pm 0.4$  (mean  $\pm$  SD) in the three vessels; the mean upper limit of this distribution oc-

TABLE 1. Total concentrations of the major plasma lipids in the portal and hepatic veins and in the hepatic artery of preruminant calves after a 17-h fast

Blood Vessels	Plasma Lipid Concentration				
	Phospholipids	Triglycerides	Total Cholesterol <sup>a</sup>	Free Cholesterol	Cholesteryl Esters
	mg/dl plasma				
Portal vein	74.4 $\pm$ 7.3	19.1 $\pm$ 1.3	56.4 $\pm$ 3.5	10.2 $\pm$ 1.3	77.6 $\pm$ 4.7
Hepatic artery	73.6 $\pm$ 9.2	13.1 $\pm$ 7.6	58.1 $\pm$ 2.3	10.3 $\pm$ 0.9	80.3 $\pm$ 3.1
Hepatic vein	71.2 $\pm$ 10.2	13.3 $\pm$ 4.2	61.5 $\pm$ 1.9	10.3 $\pm$ 0.8	86.1 $\pm$ 2.9

Values are the mean  $\pm$  SEM of duplicate analyses on plasma from four calves; plasmas from these animals were used for lipoprotein isolation by density gradient ultracentrifugation.

<sup>a</sup>Values for total cholesterol represent the sum of free and esterified cholesterol.



**Fig. 2.** A representative pattern, obtained by analytical ultracentrifugation, of the main components of the calf plasma lipoprotein spectrum from the portal vein (PV, —), hepatic artery (HA, ---), and hepatic vein (HV, - · -). LDL were analyzed at a density of 1.063 g/ml and HDL at 1.210 g/ml as described in Materials and Methods.

curred at an  $S_f$  rate of  $7.2 \pm 1.4$  in the three hepatic vessels. In one portal plasma sample, a small amount of lipoprotein material was clearly observed to sediment at a density of 1.063 g/ml. Plasma LDL concentrations were also systematically low in the portal ( $25.9 \pm 12.4$  mg/dl) and in the hepatic ( $31.3 \pm 6.2$  mg/dl) veins but higher in the hepatic artery ( $43.1 \pm 13.2$  mg/dl).

The distribution and plasma concentrations of HDL were determined by analytical ultracentrifugation at a solvent density of 1.210 g/ml (Fig. 2). The HDL class constituted the main lipoprotein component in plasma from the three hepatic vessels ( $307 \pm 55$  mg/dl) with a mean peak  $F_{1,21}$  rate of  $3.7 \pm 0.5$  in the portal vein and of  $4.2 \pm 0.6$  in the hepatic vein and hepatic artery, respectively. The mean upper limit of the distribution of total HDL occurred at an  $F_{1,21}$  of  $12.2 \pm 2.4$  in the portal vein but at  $10.6 \pm 0.7$  and  $10.4 \pm 0.4$  in the hepatic artery and in the hepatic vein, respectively. The ratio of the concentrations of  $HDL_2$  and  $HDL_3$ , defined respectively as HDL with  $F_{1,21} > 3.5$  and  $F_{1,21} < 3.5$ , varied according to the site of blood sampling; it was significantly higher ( $P < 0.05$ ) in the hepatic ( $2.48 \pm 0.77$ ) than in the portal vein ( $1.48 \pm 0.09$ ) and intermediate between these two in the hepatic artery ( $2.04 \pm 0.47$ ).

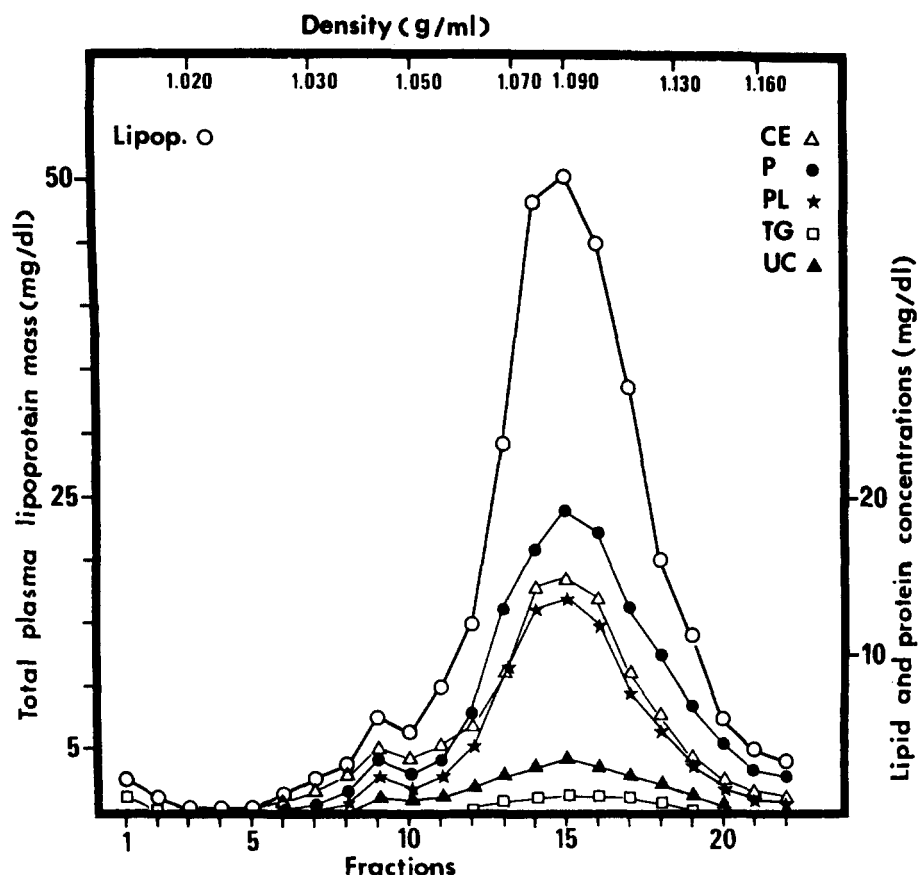
**Density gradient ultracentrifugation.** In an attempt to evaluate more precisely the distribution of lipoprotein particles as a function of their hydrated density, we first subfractionated plasma lipoproteins from the preruminant calf into 22 fractions by use of isopycnic density gradient ultracentrifugation and subsequently analyzed their lipid and protein contents (Fig. 3). This procedure allowed analysis of lipoprotein particles with hydrated densities up to 1.180 g/ml. It is noteworthy, however, that this type of gradient does not permit isolation of conventional VLDL but rather of lipoproteins with  $d < 1.018$

g/ml. The physicochemical properties and apolipoprotein content of the lipoprotein gradient subfractions presented below prompted us to define preruminant calf LDL as lipoproteins of  $d$  1.026–1.060 g/ml, light HDL ( $HDL_L$ ) as lipoproteins of  $d$  1.060–1.091 g/ml, and heavy HDL ( $HDL_H$ ) as lipoproteins of  $d$  1.091–1.180 g/ml. This nomenclature is used henceforth throughout our report.

As shown in Table 2, subfraction 1 ( $d < 1.018$  g/ml), had the highest proportion of triglyceride (44 to 47%) and the lowest of protein (16 to 18%). This fraction represented 1.1% of the total lipoproteins of  $d < 1.180$  g/ml in the portal vein and only 0.2 and 0.4% in the hepatic vein and in the hepatic artery, respectively ( $P < 0.05$ ). It is of note that, in subfractions 2–5 ( $d$  1.018–1.026 g/ml), lipoproteins were essentially absent in the fasting plasma of these calves and were undetectable in our chemical analyses (Fig. 3 and Table 2). Therefore, taking into account this fact and the compositional data reported above, lipoproteins contained in the top ( $d < 1.018$  g/ml) fraction could be considered as calf VLDL.

In subfractions 6–11 ( $d$  1.026–1.060 g/ml), the proportion of triglyceride decreased consistently (from 21 to 4%) while that of protein increased from 22 to 32% with increase in density. Cholesteryl esters predominated (30–41%); the highest proportion of this lipid typically occurred in subfractions 9 and 10 of  $d$  1.040–1.053 g/ml. Subfractions 6–11 corresponded to LDL and accounted for an average of 11% of the total calf lipoproteins of  $d < 1.180$  g/ml (33 mg/dl). No significant differences in plasma LDL concentrations between the sites of blood sampling were observed.

Subfractions 12–22 ( $d$  1.060–1.180 g/ml) corresponded to HDL and were the principal lipoprotein fractions in calf plasma. They accounted for 88–93% of the total lipoproteins (258–276 mg/dl) in plasma from the three



**Fig. 3.** Density distribution of calf plasma lipoproteins, and of their individual components (cholesteryl ester, CE ( $\Delta$ ); phospholipid, PL ( $\star$ ); triacylglycerol, TG ( $\square$ ); unesterified cholesterol, UC ( $\blacktriangle$ ); and protein, P ( $\bullet$ )) after separation in the density gradient. Total lipoprotein mass ( $\circ$ ) (mg/dl plasma) is plotted on the left ordinate, and lipid and protein concentrations (mg/dl) on the right ordinate, against density (from meniscus downwards) on the abscissa. The data are taken from portal vein plasma of one calf (representative of the four animals).

hepatic blood vessels and were distributed as a single peak with a maximum at d 1.091 g/ml (Fig. 3); their chemical compositions were distinguished by high levels of cholesteryl esters (33–15%) (Table 2).

Subfractions 12–15 (d 1.060–1.091 g/ml), which we have termed light HDL (HDL<sub>L</sub>), had elevated proportions of cholesteryl ester (33–29%) (Table 2) which were greater than those typical of human HDL<sub>2</sub> (22%). However, as in human HDL<sub>2</sub>, calf HDL<sub>L</sub> were characterized by low levels of free cholesterol (4–6%) and triglyceride (1–3%). Light HDL (133–150 mg/dl) represented 53% of total HDL.

Subfractions 16–22 (d 1.091–1.180 g/ml), which corresponded to lipoprotein fractions rich in protein (40–70%) and cholesteryl ester (29–15%), were designated as heavy HDL (HDL<sub>H</sub>). They were poor in free cholesterol (1–3%); a two- to threefold increase in triglyceride (5–6%) was, however, observed in the densest fractions

(subfractions 20–22; d 1.143–1.180 g/ml). A decrease in the proportion of phospholipid occurred (from 26 to 10%) as density increased. Calf HDL<sub>H</sub>, which constituted the denser portion of the HDL peak, represented 47% of total HDL and ranged in concentration from 108 to 147 mg/dl. Their plasma concentrations differed significantly ( $P < 0.05$ ) between the three hepatic vessels: they were highest in the portal vein (142 mg/dl), lowest in the hepatic vein (108 mg/dl), and intermediary in the hepatic artery (127 mg/dl) (Table 2).

The recovery of lipids in lipoproteins isolated by gradient density ultracentrifugation (subfractions 1–22) from 12 different plasmas (four animals and three sites of blood sampling; Table 1) was complete for free cholesterol ( $105.5 \pm 1.1\%$ ), cholesteryl ester ( $105.1 \pm 7.5\%$ ), and phospholipid ( $99.7 \pm 1.3\%$ ), but lower for triglyceride ( $75.2 \pm 8.6\%$ ). When the trace amounts of lipid in subfractions 23–28 (d 1.180–1.225 g/ml and corresponding to



TABLE 2. Chemical composition (mean percentage weight) of lipoprotein subfractions in plasma from the portal vein, hepatic vein, and artery of 3-week-old fasting, pre-ruminant calves isolated over the density gradient

	VLDL	Density Gradient Fractions																		
		LDL									HDL <sub>L</sub>									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Density Limits (g/ml)	1.018	1.026	1.030	1.035	1.040	1.046	1.053	1.060	1.068	1.076	1.086	1.091	1.091	1.091	1.091	1.091	1.091	1.091	1.091	1.091
Portal vein																				
Free cholesterol	9	8	8	7	7	7	6	6	6	5	4	4	4	3	3	3	3	3	2	1
Cholesteryl ester	16	31	35	37	40	40	39	36	33	31	30	29	29	29	27	27	26	23	19	1
Triglyceride	47	21	15	11	7	6	4	3	2	1	1	1	1	1	2	2	2	3	4	5
Phospholipid	12	18	18	18	19	20	22	25	27	27	27	27	26	24	24	22	20	20	16	11
Protein	16	22	24	27	28	29	32	33	35	38	39	40	44	44	44	48	53	60	64	68
Lipoprotein concentration (mg/dl) <sup>a</sup>	3.6	1.6	2.3	3.7	6.4	7.5	11.9	17.3	28.0	39.3	48.7	45.7	45.7	38.5	38.5	24.4	14.8	9.0	6.1	4.0
% of Total lipoproteins	± 0.8	± 0.4	± 0.4	± 0.7	± 1.3	± 1.3	± 4.1	± 6.0	± 5.9	± 8.8	± 11.0	± 12.2	± 11.2	± 11.2	± 11.2	± 4.0	± 4.3	± 2.1	± 1.0	± 0.7
(d < 1.180 g/ml)	1.1	0.5	0.7	1.2	2.0	2.4	3.8	5.5	9.0	12.6	15.6	14.6	12.3	7.8	4.7	2.9	2.0	1.3	1.3	1.3
Hepatic vein																				
Free cholesterol	8	10	9	9	7	7	7	6	6	6	5	4	4	4	3	3	2	2	2	1
Cholesteryl ester	16	32	36	37	40	41	36	33	31	30	30	29	29	29	28	27	23	21	20	13
Triglyceride	46	17	13	10	6	5	3	2	1	1	1	1	1	1	1	1	2	3	4	6
Phospholipid	13	19	19	19	20	20	22	25	29	28	28	28	27	26	24	20	20	18	16	12
Protein	17	22	23	25	27	27	32	33	34	36	36	38	39	42	45	52	55	58	58	68
Lipoprotein concentration (mg/dl) <sup>a</sup>	0.5	1.6	2.3	3.5	6.9	8.0	11.0	22.3	33.6	44.4	49.4	49.4	42.5	29.8	16.4	9.6	5.1	4.2	3.2	3.2
% of Total lipoproteins	± 0.5	± 0.6	± 0.7	± 0.8	± 1.5	± 1.5	± 2.4	± 6.2	± 7.7	± 8.8	± 8.1	± 8.1	± 8.9	± 7.2	± 4.2	± 2.3	± 0.9	± 0.3	± 0.6	± 0.6
(d < 1.180 g/ml)	0.2	0.5	0.8	1.2	2.3	2.7	3.7	7.6	11.4	15.1	16.8	14.4	10.1	5.6	3.3	1.7	1.4	1.1	1.1	1.1
Hepatic artery																				
Free cholesterol	9	11	10	9	7	7	6	6	5	4	4	4	4	3	3	3	2	2	2	1
Cholesteryl ester	17	30	34	37	41	40	38	32	31	31	30	29	29	28	26	24	20	18	15	15
Triglyceride	44	19	14	10	6	5	4	3	2	1	1	1	1	1	1	2	3	4	5	6
Phospholipid	12	19	19	19	19	20	21	25	27	28	27	26	25	23	21	17	13	8	7	8
Protein	18	21	23	25	27	28	31	34	35	36	38	41	43	47	50	57	63	70	73	70
Lipoprotein concentration (mg/dl) <sup>a</sup>	1.1	1.3	2.3	3.3	7.6	6.2	9.7	15.5	29.1	47.5	49.5	44.8	33.1	19.7	13.5	7.1	5.2	3.5	3.5	3.5
% of Total lipoproteins	± 0.1	± 0.4	± 0.6	± 0.7	± 2.5	± 1.7	± 1.4	± 1.2	± 4.1	± 9.4	± 7.6	± 9.3	± 11.7	± 8.4	± 6.2	± 1.9	± 2.0	± 2.0	± 0.7	± 0.7
(d < 1.180 g/ml)	0.4	0.4	0.8	1.1	2.5	2.1	3.2	5.2	9.7	15.8	16.5	14.9	11.0	6.6	4.5	2.4	1.7	1.2	1.2	1.2

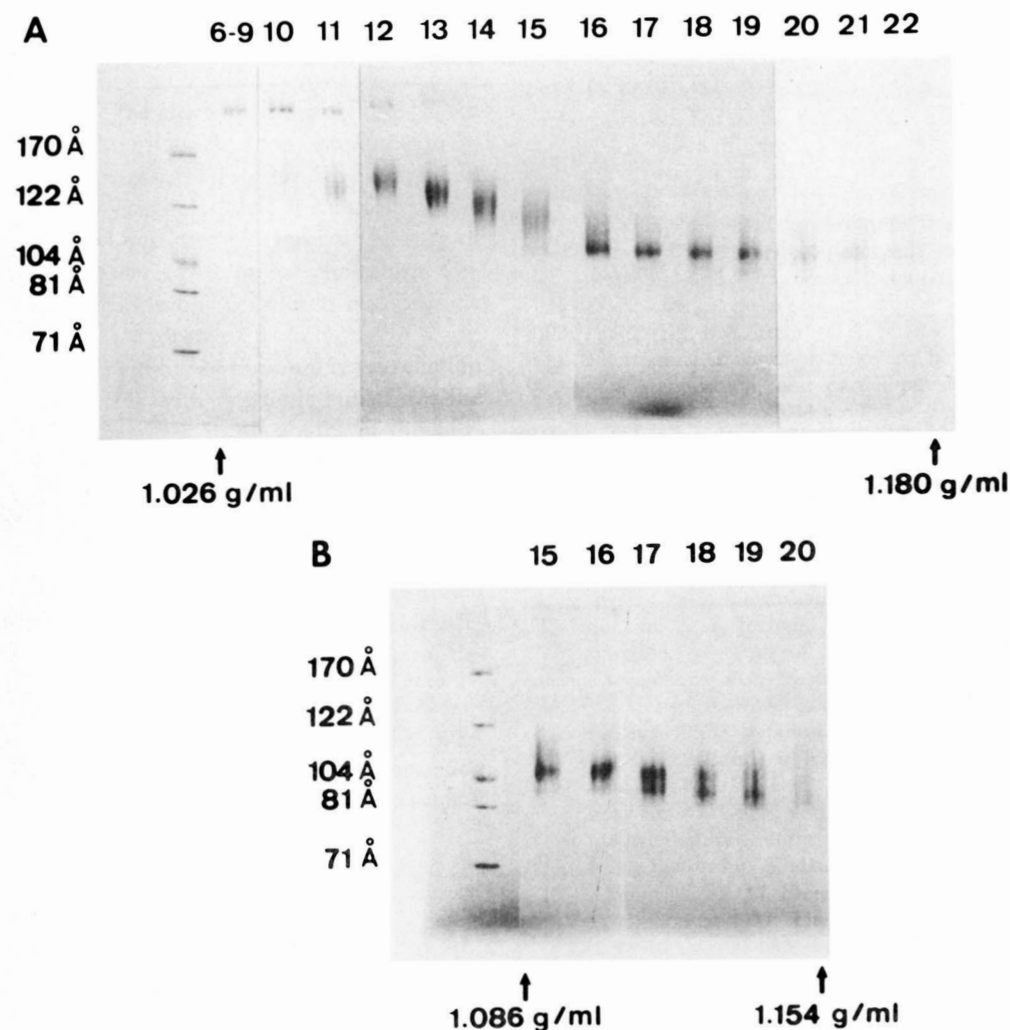
Amounts of LDL (d 1.018-1.028 g/ml, fractions 2-5) were insufficient to allow chemical analysis and have been omitted.

<sup>a</sup>Values are the mean ± SEM of duplicate analyses of plasma from four calves that were used for lipoprotein isolation by density gradient ultracentrifugation. The range of the standard error of the mean for determination of each component was: cholesterol, 0.2-0.4%; free cholesterol, 0.1-0.3%; triglyceride, 0.2-1.0%; phospholipid, 0.4-0.8%; and protein, 0.3-0.7%.

the bottom subfractions) were taken into account, total recovery amounted to  $107.1 \pm 1.3\%$  for free cholesterol,  $105.1 \pm 7.5\%$  for cholesteryl ester,  $105.1 \pm 1.2\%$  for phospholipid, and  $93.9 \pm 7.9\%$  for triglyceride.

**Electrophoretic analyses of native plasma lipoproteins.** Electrophoresis of native lipoprotein subfractions, isolated from the density gradients and corresponding to the four animals and three vessels, was performed in nondenaturing gels in order to evaluate the heterogeneity and size distribution of lipoprotein particles (Fig. 4). However, lipoprotein concentrations were too low to allow such analyses in subfractions 1–5 of  $d < 1.026$  g/ml; in view of the small amounts available, subfractions 6–8 ( $d$  1.026–1.040 g/ml) were pooled.

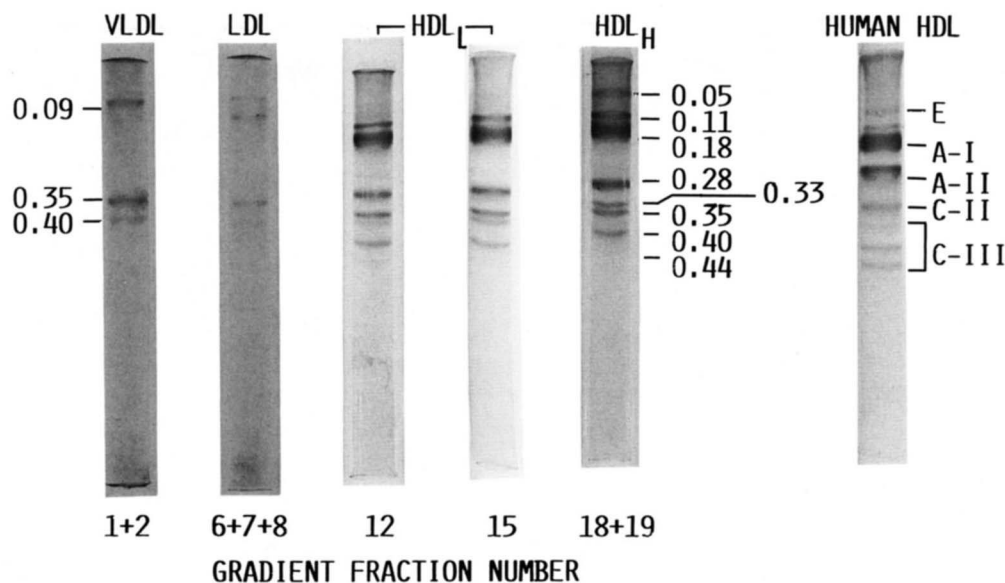
Subfractions 6–12, corresponding to the density interval 1.026–1.068 g/ml, displayed a major band ranging in diameter from 193 to 207 Å according to the animal considered and corresponding to classical LDL particles (5); this pattern was observed in all samples. In two animals, the species of diameter 193–207 Å was detected up to a density of 1.076 g/ml (i.e., as a component of subfraction 13). A second band, ranging in size from 150 to 130 Å, was, however, present in subfractions 11–14 ( $d$  1.053–1.086 g/ml). Therefore, subfractions 11–13 ( $d$  1.053–1.076 g/ml) simultaneously contained lipoproteins with diameters typical of LDL and HDL. In addition, traces of this latter component were occasionally detected in fraction 10 ( $d$  1.046–1.053 g/ml).



**Fig. 4.** Polyacrylamide gradient gel electrophoresis of native lipoprotein gradient subfractions from portal vein plasma. The number of each subfraction is indicated at the top of each part of the figure (see Fig. 3 and Table 2 for corresponding densities). The gel slabs contained a continuous gradient of 4–30% acrylamide (from top to bottom). A set of standard marker proteins was loaded in the first lane on the left; the standards and their Stokes diameters are, respectively; thyroglobulin 170 Å; ferritin 122 Å; catalase 104 Å; lactic dehydrogenase 81 Å; bovine serum albumin 71 Å. Upper section, 4A: Subfractions 6–22 from a calf displaying single size species in the density range 1.086–1.154 g/ml (subfractions 15–20). Lower section, 4B: Subfractions 15–20 from a calf displaying two distinct size species over the same density range.







**Fig. 6.** Representative tetramethylurea-soluble apolipoprotein profiles from lipoprotein subfractions isolated by density gradient ultracentrifugation of calf plasma (portal vein). Electrophoresis was performed according to the method of Kane (37). A pattern from human HDL is shown at right.  $R_f$  values of the major components are indicated at left of the pattern for VLDL (fraction 1) and at right for HDL<sub>H</sub> (fractions 18 + 19). The correspondence between gradient fraction number and lipoprotein density class is indicated in Table 2.

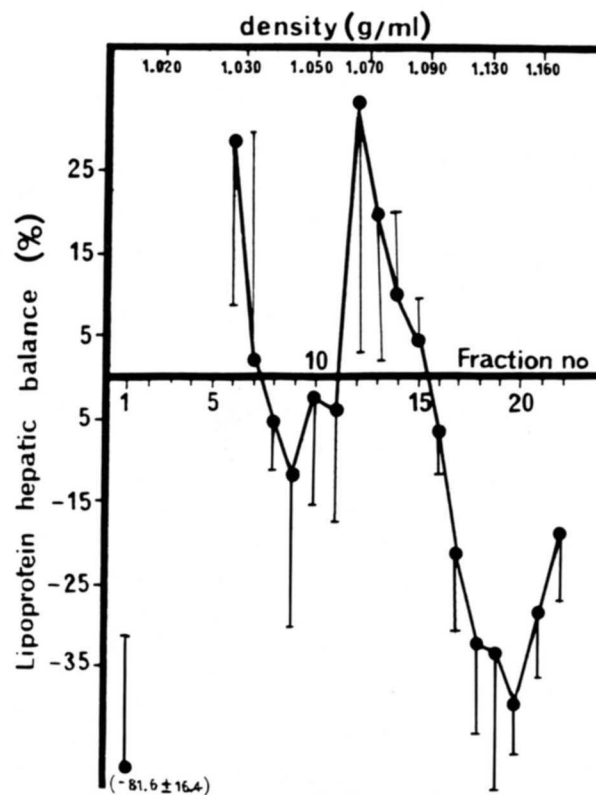
proteins of calf plasma, i.e., either the lighter lipoproteins of  $d < 1.024$  g/ml (VLDL) or the denser lipoproteins of  $d$  1.131–1.180 g/ml (HDL<sub>H</sub>), the latter containing up to 6% of triglyceride.

**Hepatic balance of lipoprotein subfractions.** Mean blood flows in the portal vein and in the hepatic artery of fasting calves amounted to  $2,467 \pm 147$  ml/min (48 ml/min per kg body wt) and  $287 \pm 17$  ml/min (5.6 mg/min per kg body wt), respectively. Mean hepatic arterial blood flow represented 10.4% of total afferent blood flow in the liver. The corresponding plasma flows averaged  $1,780 \pm 216$  ml/min (34.6 ml/min per kg body wt) in the portal vein and  $207 \pm 24$  ml/min (4.03 ml/min per kg body wt) in the hepatic artery.

The hepatic balance for the 22 lipoprotein subfractions (expressed as flow % for each subfraction presented to the liver) is illustrated schematically in **Fig. 7**. For subfraction 1 (VLDL), it was markedly negative ( $-82.6\%$ ) and corresponded to a net uptake of  $1.06 \pm 0.24$  mg/min per kg body wt ( $P < 0.01$ ). For subfractions 6–11 (LDL), large variability in the hepatic balance between animals was detected. Similar measurements led to an apparent uptake of  $0.44 \pm 1.06$  mg/min per kg body wt for subfractions 8–11.

For subfractions 12–15, which corresponded to HDL<sub>L</sub>, the hepatic balance was significantly positive ( $P < 0.05$ ; 5–30%), the absolute amount of these subfractions produced by the liver representing  $5.92 \pm 5.53$  mg/min per kg body wt.

For subfractions 16–22, which corresponded to HDL<sub>H</sub>, the hepatic balance was typically negative ( $-6.0$  to



**Fig. 7.** Hepatic balance is expressed as

$$\frac{C_{HV}(F_{PV} + F_{HA}) - (C_{PV}F_{PV} + C_{HA}F_{HA})}{C_{PV}F_{PV} + C_{HA}F_{HA}} \times 100.$$

for each lipoprotein gradient fraction in fasting preruminant calves ( $n = 4$ ) (for details, see Materials and Methods). Insufficient amounts were present in gradient fractions 2–5 to permit analysis, and these fractions have, therefore, been omitted.



– 40.4%), involving a net loss of  $11.34 \pm 4.47$  mg/min per kg body wt ( $P < 0.01$ ); this finding was in marked contrast to the net gain observed for subfractions 12–15 ( $P < 0.01$ ).

## DISCUSSION

As a functional monogastric mammal, the preruminant calf presents considerable interest as a species in which to evaluate the specific role of key organs in lipoprotein homeostasis in vivo. Thus, the major blood and lymphatic vessels are amenable to surgical cannulation and electromagnetic determination of flow rates (27), and in addition, relatively large volumes of blood or lymph may be removed without undue stress to the animal. Moreover, its size makes it considerably more suitable for such experimentation than its adult counterparts, the cow and steer. In view of our rudimentary knowledge of lipid transport in the preruminant calf (2, 3, 12, 24–26), we have determined the principal physicochemical and hydrodynamic features of the various lipoproteins as a function of their density distribution in the portal vein, hepatic artery, and hepatic vein. Furthermore, as a consequence of the simultaneous determination in vivo of lipoprotein fluxes in the afferent and efferent hepatic blood vessels, we provide new insight into the role of the liver in the metabolism of individual lipoprotein particle species.

We shall first consider the physicochemical properties and apolipoprotein contents of calf plasma lipoproteins. The chemical composition of fasting calf VLDL ( $d < 1.018$  g/ml; fraction 1) was intermediate between that of the lactating dairy cow (9), and that reported for newborn calf and steer VLDL (3, 12), particularly with respect to both triglyceride and protein contents. These discrepancies may, in part, arise from the different densities and methodologies used for the isolation of VLDL. In addition, it is notable that the  $d < 1.018$  g/ml VLDL isolated in the present study did not appear to be contaminated with lipoproteins of intermediate density, the latter being almost undetectable over the density range 1.018–1.026 g/ml (Table 2).

Two species of apolipoprotein B-like proteins dominated the protein moiety of VLDL, each migrating as a single band upon electrophoresis in SDS-polyacrylamide gradient gels (Fig. 5) and displaying molecular weights of  $\sim 520,000$  and  $\sim 265,000$ , respectively. These findings clearly document, for the first time, the presence of an  $M_r$  520,000 counterpart to the human B-100 protein (40) in bovine triglyceride-rich lipoproteins. Furthermore, this B-100-like component was also detected in calf LDL subfractions (6–11;  $d$  1.026–1.060 g/ml). Earlier reports in both the calf and adult bovine have either failed to detect this apolipoprotein (15, 42), or have alluded to its presence

on the basis of its solubility properties (3), or alternatively have identified the apoB-like components as proteins with sizes in the range of  $\sim 200,000$  to  $\sim 370,000$  (13, 42–44). The apparent  $M_r$  value of 520,000 determined herein closely resembles that of apoB-100 in other mammals, including pig (45), mouse (46), common marmoset (47), and man (40), but is greater than that seen in the rat (as B<sub>h</sub>; ref. 48). The lower  $M_r$  form ( $\sim 265,000$ ) in the calf may be tentatively considered as a B-48 counterpart (40): this form may correspond to the  $M_r$  220,000 protein detected earlier in bovine VLDL which Grummer et al. (13) found to predominate over a second band with  $M_r$  290,000. As the B-48-like protein occurred primarily in portal vein plasma (Fig. 5), it is interesting to speculate that it may arise by direct secretion of nascent intestinal VLDL into this vessel (see below). Further studies are, however, required to evaluate the tissular origin of the calf B-100- and B-48-like proteins.

At least six, nonB apolipoproteins of  $M_r < 100,000$  were typically detected in calf VLDL. Of particular interest was the 42,000 dalton protein, which was also present in lipoproteins of  $d > 1.131$  g/ml. The presence of this component in our SDS gel patterns corresponded to that of the  $R_f$  0.05–0.09 band seen upon electrophoretic analysis in alkaline urea gels (Fig. 6). Taken together, the size and density distribution of this protein suggest that it is the calf counterpart to human apoA-IV ( $M_r$  46,000; refs. 49, 50). Indeed, our observations are not inconsistent with an intestinal synthesis and lymphatic secretion of this protein as a component of intestinal chylomicrons and VLDL, with subsequent transfer to plasma lipoproteins of very high density ( $d > 1.17$  g/ml) (51, 52). The hepatic synthesis of apoA-IV in the calf, as in the rat (53, 54), cannot, however, be excluded on the basis of our present data, although no marked differences were evident in the amounts of the 42,000 dalton protein in VLDL from the hepatic vein and portal vein. The bovine apoA-IV-like component has been previously detected in triglyceride-rich lipoproteins and LDL separated by gel filtration chromatography from the plasma of lactating and nonlactating cows ( $M_r$  46,000; ref. 13) and in the unusual intermediate density lipoproteins of the lactating cow (15).

We failed to detect a bovine counterpart to human apoE ( $M_r$  34,200; ref. 55), although such a component was tentatively identified as a band of  $M_r$  36,000 in the triglyceride-rich lipoproteins and LDL of lactating cows (13), and in the VLDL and IDL of cholesterol-fed calves (42); its absence in the present studies may be related to our use of fasting conditions.

While C apolipoproteins were lacking in the triglyceride-rich lipoproteins of lactating cows (13), we detected up to five bands with migration characteristics considered typical of these small proteins in our two electrophoretic systems (Fig. 5 and 6). These findings are consistent with those of Forte, Bell-Quint, and Cheng (3) and Puppione

et al. (15); moreover, certain of these apoC-like peptides have been purified from either the whole plasma or HDL of cows (56–60) and shown to display either activating or inhibitory effects on bovine milk lipoprotein lipase activity (56, 58–60). ApoA-I ( $M_r$  28,000) was a minor component of calf VLDL (Fig. 5) as reported earlier (3).

Quantitatively, VLDL amounted to 5% or less of the total lipoproteins of  $d < 1.180$  g/ml in plasma from each of the three sites of sampling when determined by analytical ultracentrifugation, and even less ( $\sim 1\%$ ) by chemical analysis (Table 2). This discrepancy may, at least in part, arise from technical difficulties encountered in quantifying very low concentrations of a highly disperse particle population by schlieren analysis. Such low VLDL levels ( $< 20$  mg/dl) have been described earlier (3), but may increase markedly (up to 60 mg/dl) when calves are fed a high fat milk replacer ( $\geq 20\%$  dry matter) during the growing period (24).

Particles with diameters (193–207 Å) typical of LDL were distributed over the density range 1.026–1.076 g/ml (Fig. 4). However, only subfractions 6–9 ( $d$  1.026–1.046 g/ml) contained a unique and homogeneous LDL size subspecies. The major LDL subfraction (9,  $d$  1.040–1.046 g/ml) was comparable to that of other mammals in its chemical composition (2, 5), in its  $\beta$ -mobility, and its chemical composition, being enriched in cholesteryl esters but poor in triglycerides and free cholesterol (Table 2). Furthermore, the protein moieties of these particles were dominated by the apoB-like proteins. Small amounts of apolipoproteins of lower molecular weight were also found, of which bovine apoA-I was the most prominent. Our data suggest then that apoB-rich LDL particles in the calf may also carry apoA-I, a finding consistent with the presence of both apoB and apoA-I in LDL separated from bovine plasma by affinity chromatography (44).

In addition to their content of particles typical of LDL, subfractions 10 and 11 ( $d$  1.046–1.060 g/ml) also presented a faintly stained band indicative of the presence of HDL of 140–150 Å diameter (Fig. 4); these particles predominated however in subfraction 12 ( $d$  1.060–1.068 g/ml) in which LDL were the minor particle-size species. We cannot, however, exclude the possibility that some degree of cross-contamination occurred between adjacent subfractions in this density region given the elevated concentrations of HDL particles in subfraction 12. Nonetheless, in the adult bovine (43), particles with the physical characteristics of LDL and of HDL coexist and overlap in distribution over the density range 1.046–1.068 g/ml in the calf. Given this mixture of LDL and HDL particles, it would clearly be misleading to compare data on the chemical composition of calf LDL of  $d$  1.006–1.063 g/ml (3, 24) with those of the LDL subfractions isolated in the present study. Nonetheless, the particle size of the former LDL, as determined by electron microscopic analysis (194 Å) by

Forte et al. (3), and those presently described (193–207 Å) are alike.

The high density lipoproteins of the preruminant calf were the major plasma particles ( $\sim 90\%$  of the total  $d < 1.180$  g/ml substances), in confirmation of earlier studies (3, 24, 42). They presented as two distinct populations on the basis of their physicochemical properties, and we have termed them light (HDL<sub>L</sub>) and heavy (HDL<sub>H</sub>) HDL. Light HDL were distributed over the density interval from 1.053 to 1.091 g/ml (subfractions 11–15) and ranged in particle size from 150 to 130 Å (Figs. 3 and 4). However, as particles of HDL-size were the major components of gradient subfractions of  $d > 1.060$  g/ml, we have used this density as the cutoff point between LDL and HDL<sub>L</sub> for present purposes (Table 2). These HDL<sub>L</sub> particles were distinct in their elevated cholesteryl ester and low triglyceride contents.

The heavy HDL subpopulation, of  $d$  1.091–1.180 g/ml, accounted for almost half of total plasma calf HDL and, like light HDL, was rich in cholesteryl esters. By contrast, they contained more protein and triglyceride (typically  $> 1\%$  and increasing in parallel with density to 6%) but less phospholipid and free cholesterol than their lighter counterparts. Heavy HDL had sizes of 93–120 Å, a finding consistent with their lower proportions of core lipids ( $\sim 19$ –30%) as compared to light HDL ( $\sim 30$ –36%).

The protein moieties of light and heavy HDL could not be differentiated, with the exception of the appearance of the  $M_r$  42,000 apoA-IV-like protein in the latter (Fig. 5). In addition, the relative proportions of the various apoC-like polypeptides (56, 57, 59, 60) varied among the different subfractions of both light and heavy HDL as indicated by the variation in their relative staining intensities across this density range (Figs. 5 and 6).

Comparison of the physicochemical properties of calf light and heavy HDL with the two major density subpopulations of human HDL separated by the same procedure revealed several marked dissimilarities. Thus, elevated proportions of cholesteryl esters and lower contents of triglyceride in calf HDL<sub>L</sub> differentiated them from human HDL<sub>2</sub> (Table 5, ref. 29) while the major subfractions of HDL<sub>H</sub> (16–18,  $d$  1.091–1.131 g/ml, representing 75% of plasma HDL<sub>H</sub> levels) were similarly of higher cholesteryl ester content than human HDL<sub>3</sub> (29). As anticipated, such higher contents of core lipid were reflected in large particle diameters in calf HDL (HDL<sub>L</sub>, 110–150 Å; HDL<sub>H</sub>, 93–120 Å) as compared to their human counterparts (HDL<sub>2</sub>, 72–108 Å; HDL<sub>3</sub>, 54–90 Å, ref. 29). In all probability, the mechanistic basis for the high cholesteryl ester content of calf HDL lies, on the one hand, in the low plasma cholesteryl ester transfer activity in bovine species, which approximates 10–20% of that in human plasma (61) and, on the other hand, in the low plasma levels of cholesteryl ester acceptor particles,

notably of VLDL and LDL. Under such conditions, cholesteryl esters formed in HDL via the LCAT reaction appear to be limited in their ability to transfer to other, less dense particles. The core of the HDL particles must therefore expand to accommodate such esterified cholesterol, thereby accounting for the role of HDL as the major vehicle for cholesteryl ester transport in the calf (2). Indeed, and as reported earlier, the HDL of both the cow and steer are also rich in cholesteryl esters (2-4, 16) and thus appear to play a major role in the plasma transport of this hydrophobic lipid.

On a qualitative basis, the physicochemical properties of lipoprotein subfractions in plasma from the portal vein, hepatic vein, and hepatic artery were indistinguishable. By contrast, significant differences were found in the absolute concentrations of individual subfractions at the three sites of sampling and these were reflected in determinations of lipoprotein fluxes across the liver in vivo. Thus, measurements of HDL flux indicated a net hepatic production of light HDL and a net uptake of heavy HDL in our fasting calves (Fig. 7). These findings are consistent with the hypothesis that either heavy HDL are transformed into lighter particles (i.e., HDL<sub>L</sub>) as they traverse the liver and appear in the hepatic vein, or that heavy HDL are preferentially taken up by the liver with concomitant production of lighter HDL, or a combination of both. Such findings tend to contradict current hypotheses of hepatic HDL metabolism (62), at least in those mammals whose plasma exhibits high cholesteryl ester transfer activity (61). Alternatively, however, light HDL may be initially secreted as denser, smaller particles. We speculate that such HDL may undergo rapid and extensive conversion into lighter particles as a result of potentially elevated LCAT activity in the hepatic vein. The present data do not allow us to interpret these hypotheses further; moreover, the relevance to these findings of the low hepatic lipase activity in bovine species (63) remains indeterminate.

The three- to sixfold higher level of VLDL ( $d < 1.018$  g/ml) in the portal vein as compared to those in the hepatic artery and hepatic vein (Table 2) was unexpected and suggests not only a direct secretion of intestinal VLDL into this vessel but also an intense hepatic clearance ( $\sim 80\%$ ) of VLDL. Such clearance appeared largely independent of nutritional state since similar results were obtained with the same animals during the postabsorptive period (D. Bauchart, D. Durand, and M. J. Chapman, unpublished observations). Experiments are now in progress to quantify the relative roles of the lymphatic intestinal duct and the portal vein in the transport of nascent bovine intestinal VLDL.

Low density lipoproteins ( $d$  1.026-1.060 g/ml) represented approximately 10% of total  $d < 1.180$  g/ml lipoproteins in fasting plasma from the three hepatic vessels. The slight differences in their respective concentrations led to

an insignificant negative balance suggesting that the sum of the uptake, formation, and secretion processes across the liver were equivalent under our nutritional conditions. Some precision was, however, lacking in these measurements as there was a small contribution (probably  $< 10\%$ ) of HDL to this LDL fraction, and, conversely, of LDL to the light HDL subpopulation. These findings demonstrated the inadequacy of the 1.063 g/ml density cutoff point for LDL and HDL in the calf, as indeed we have previously shown in other mammals (2, 5). Alternative methodologies are therefore required for complete separation of these two lipoprotein classes (43).

In conclusion, our in vivo determination of lipoprotein balance across the liver has provided new insight on the role of this organ in the uptake, intravascular transformation, and formation of VLDL and HDL in a monogastric mammal. ■■

This work was supported by INRA Grant no. 4432 to D. B., D. D., M. J. C., and P. M. L. The authors are grateful to Mr. J. Lefavre for animal surgery, to Mrs. Marinett Martinaud and Mrs. Christiane Legay for skillful technical assistance, and Mr. R. Souchet and Mr. C. Léoty for the excellent maintenance and care of the animals.

Manuscript received 2 December 1988 and in revised form 10 March 1989.

## REFERENCES

1. Noble, R. C. 1978. Digestion, absorption and transport of lipids in ruminant animals. *Prog. Lipid Res.* **17**: 55-91.
2. Chapman, M. J. 1980. Animal lipoproteins: chemistry, structure and comparative aspects. *J. Lipid Res.* **21**: 789-853.
3. Forte, T. M., J. J. Bell-Quint, and F. Cheng. 1981. Lipoproteins of fetal and new-born calves and adult steer: a study of developmental changes. *Lipids.* **16**: 240-245.
4. Puppione, D. L. 1983. Bovine serum lipoproteins. In *CRC Handbook of Electrophoresis*. Volume IV. Lipoprotein studies of non-human species. L. A. Lewis and H. K. Naito, editors. CRC Press Inc, Boca Raton, FL. 185-202.
5. Chapman, M. J. 1986. Comparative analysis of mammalian plasma lipoproteins. Plasma lipoproteins: preparation, structure and molecular biology. *Methods Enzymol.* **128**: 70-143.
6. Mills, G. L., and C. E. Taylaur. 1971. The distribution and composition of serum lipoproteins in eighteen animals. *Comp. Biochem. Physiol.* **40**: 489-501.
7. Jonas, A. 1972. Physicochemical properties of bovine serum high density lipoprotein. *J. Biol. Chem.* **247**: 7767-7772.
8. Jonas, A. 1975. Isolation and partial characterization of the major apolipoprotein component of bovine serum high density lipoprotein. *Biochim. Biophys. Acta.* **393**: 460-470.
9. Stead, D., and V. A. Welch. 1975. Lipid composition of bovine serum lipoproteins. *J. Dairy Sci.* **58**: 122-127.
10. Swaney, J. B. 1980. Characterization of the high density lipoprotein and its major apoprotein from human, canine, bovine and chicken plasma. *Biochim. Biophys. Acta.* **617**: 489-496.
11. Raphael, B. C., P. S. Dimick, and D. L. Puppione. 1973. Lipid characterization of bovine serum lipoproteins



- throughout gestation and lactation. *J. Dairy Sci.* **56**: 1025-1032.
12. Quincey, D., D. Le Goff, J. Fresnel, and A. Nouvelot. 1987. Qualitative and quantitative alterations of bovine serum lipoproteins with ageing. *Comp. Biochem. Physiol.* **88**: 929-937.
13. Grummer, R. R., C. A. Meacham, W. L. Hurley, and C. L. Davis. 1987. Apolipoprotein composition of bovine lipoproteins isolated by gel filtration chromatography. *Comp. Biochem. Physiol.* **88**: 1163-1174.
14. Puppione, D. L. 1978. Implications of unique features of blood lipid transport in the lactating cow. *J. Dairy Sci.* **61**: 651-659.
15. Puppione, D. L., S. T. Kunitake, R. Hamilton, M. L. Phillips, V. N. Schumaker, and L. D. Davis. 1982. Characterization of unusual intermediate density lipoproteins. *J. Lipid Res.* **23**: 283-290.
16. Puppione, D. L., S. T. Kunitake, M. L. Toomey, E. Loh, and V. N. Schumaker. 1982. Physicochemical characterization of ten fractions of bovine alpha lipoproteins. *J. Lipid Res.* **23**: 371-379.
17. Glascock, R. F., and V. A. Welch. 1974. Contribution of the fatty acids of three low-density serum lipoproteins to bovine fat milk. *J. Dairy Sci.* **58**: 122-127.
18. Puppione, D. L., G. M. Forte, A. V. Nichols, and E. H. Strisower. 1970. Partial characterization of serum lipoproteins in the density interval 1.04-1.06 g/ml. *Biochim. Biophys. Acta.* **202**: 392-395.
19. Christie, W. W. 1978. The composition, structure and function of lipids in the tissues of ruminant animals. *Prog. Lipid Res.* **17**: 111-205.
20. Harfoot, C. G. 1978. Lipid metabolism in the rumen. *Prog. Lipid Res.* **17**: 21-38.
21. Reid, I. M., and C. J. Roberts. 1983. Subclinical fatty liver in dairy cows. Current research and future prospects. *Irish Vet. J.* **37**: 104-110.
22. Bauchart, D., B. Aurousseau, and E. Auclair. 1985. Addition of sorbitol to a milk substitute for veal calves. I. Effects on health, growth and feed conversion. *Reprod. Nutr. Dev.* **25**: 399-410.
23. Bauchart, D., B. Aurousseau, E. Auclair, and A. Labarre. 1985. Addition of sorbitol to a milk substitute for veal calves. II. Effects on plasma, liver and muscle lipids. *Reprod. Nutr. Dev.* **25**: 411-425.
24. Bauchart, D., and D. Leveux. 1985. Lipoprotéines plasmatiques du veau préruminant. *Reprod. Nutr. Dev.* **25**: 243-250.
25. Durand, D., D. Bauchart, and J. Lefaivre. 1984. In vivo hepatic balance of lipids and glucose in the calf: effects of sorbitol intake. *Can. J. Anim. Sci.* **64**: 238-239.
26. Durand, D., and D. Bauchart. 1986. Variations nyctémérales de la lipémie et de la glycémie au niveau des voies afférentes et efférentes du foie chez le veau préruminant. *Reprod. Nutr. Dev.* **26**: 371-372.
27. Durand, D., D. Bauchart, J. Lefaivre, and J. P. Donnat. 1988. Method for continuous measurement of blood metabolite hepatic balance in conscious prerinant calves using catheters and electromagnetic flowmetry. *J. Dairy Sci.* **71**: 1632-1642.
28. Zilversmit, D. B. 1969. Chylomicrons. In *Structural and Functional Aspects of Lipoproteins in Living Systems*. E. Tria and A. M. Scanu, editors. Academic Press, London and New York. 329-368.
29. Chapman, M. J., S. Goldstein, D. Lagrange, and P. M. Laplaud. 1981. A density gradient ultracentrifugal procedure for the isolation of the major lipoprotein classes from human serum. *J. Lipid Res.* **22**: 339-358.
30. Laplaud, P. M., L. Beaubatie, and D. Maurel. 1980. A spontaneously seasonal hypercholesterolemic animal: plasma lipid and lipoproteins in the European badger (*Meles meles* L.). *J. Lipid Res.* **21**: 724-738.
31. Anderson, D. W., A. V. Nichols, J. M. Forte, and F. T. Lindgren. 1977. Particle distribution of human serum high density lipoproteins. *Biochim. Biophys. Acta.* **493**: 55-68.
32. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
33. Fossati, P., and L. Prencipe. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.* **28**: 2077-2080.
34. Takayama, M., S. Itoh, T. Nagasaki, and I. Tonimizu. 1977. A new enzymatic method for determination of serum choline-containing phospholipids. *Clin. Chim. Acta.* **79**: 93-98.
35. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies of the proteins in human plasma very low density lipoproteins. *J. Biol. Chem.* **244**: 5687-5694.
36. Irwin, D., P. A. O. Looney, E. Quinet, and G. Vahouny. 1984. Application of SDS gradient polyacrylamide slab gel electrophoresis to analysis of apolipoprotein mass and radioactivity of rat apolipoproteins. *Atherosclerosis.* **53**: 163-172.
37. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53**: 350-364.
38. Brockman, R. P., and E. N. Bergman. 1975. Quantitative aspects of insulin secretion and its hepatic and renal removal in sheep. *Am. J. Physiol.* **229**: 1338-1343.
39. Mann, H. B., and D. R. Whitney. 1947. On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.* **18**: 50-60.
40. 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.
41. Stead, O., and V. A. Welch. 1976. Immunological properties of bovine serum lipoproteins and chemical analysis of their protein moieties. *J. Dairy Sci.* **51**: 1-8.
42. Beynen, A. C., and L. G. M. Van Gils. 1983. Composition of serum lipoproteins in veal calves fed a milk replacer supplemented with cholesterol. *Nutr. Rep. Int.* **27**: 587-597.
43. Cordle, S. R., R. A. Clegg, and S. J. Yeaman. 1985. Purification and characterization of bovine lipoproteins: resolution of high density and low density lipoproteins using heparin-Sepharose chromatography. *J. Lipid Res.* **26**: 721-725.
44. Cordle, S. R., W. J. Koper, and S. J. Yeaman. 1986. Isolation and characterization of bovine lipoproteins: incubation with hepatic lipase and isolated adrenal zona fasciculata cells. *Biochem. Soc. Trans.* **13**: 879-880.
45. Chapman, M. J., and P. Forgez. 1985. Lipid transport systems: some recent aspects in swine, cattle and trout during development. *Reprod. Nutr. Dev.* **25**: 217-226.
46. 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.
47. Forgez, P., M. J. Chapman, and G. L. Mills. 1984. 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.



48. 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.
49. Weisgraber, K. H., T. P. Bersot, and R. W. Mahley. 1978. Isolation and characterization of an apolipoprotein from the  $d < 1.006$  lipoproteins of human and canine lymph homologous with the rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85**: 287-292.
50. Utermann, G., and U. Beisiegel. 1979. Apolipoprotein A-IV: a protein occurring in human mesenteric lymph chylomicrons. *Eur. J. Biochem.* **99**: 333-343.
51. Green, P. H. B., R. M. Glickman, J. W. Riley, and E. Quinet. 1980. Human apolipoprotein A-IV, intestinal origin and distribution in plasma. *J. Clin. Invest.* **65**: 911-919.
52. Roheim, P. S., J. G. Delamatre, L. Dory, M. Lefevre, and G. H. Sloop. 1986. ApoA-IV metabolism and possible physiological role. In *Atherosclerosis VII*. N. H. Fidge and P. I. Nestel, editors. Elsevier, 295-298.
53. Windmueller, H. G., and H. G. Wu. 1981. Biosynthesis of plasma apolipoproteins by rat small intestine without dietary or biliary fat. *J. Biol. Chem.* **256**: 3012-3016.
54. Ghiselli, G., H. B. Brewer, Jr., and H. G. Windmueller. 1982. Apolipoprotein A-I isoprotein synthesis by the perfused rat liver. *Biochem. Biophys. Res. Commun.* **107**: 144-149.
55. Mahley, R. W., T. L. Innerarity, S. C. Rall, and K. H. Weisgraber. 1984. Plasma lipoproteins: apolipoprotein structure and function. *J. Lipid Res.* **25**: 1277-1294.
56. Lim, C. I., and A. M. Scanu. 1976. Apolipoproteins of bovine high density lipoproteins: isolation and characterization of the small-molecular-weight components. *Artery*. **2**: 483-496.
57. Patterson, B. W., and A. Jonas. 1980. Bovine apolipoproteins C. I. Isolation and spectroscopic investigations of the phospholipid binding properties. *Biochim. Biophys. Acta*. **619**: 572-586.
58. Patterson, B. C., and A. Jonas. 1980. Bovine apolipoproteins C. II. Isolation and partial physicochemical characterization of complexes with L-dimyristoyl phosphatidylcholine. *Biochim. Biophys. Acta*. **619**: 587-603.
59. Clegg, R. A. 1978. Bovine serum apolipoprotein effectors of milk lipase. *Biochem. Soc. Trans.* **6**: 1207-1210.
60. Astrup, H. N., and G. Bengtsson. 1982. Activator proteins for lipoprotein lipase from bovine plasma: preparation by adsorption to Intralipid. *Comp. Biochem. Physiol.* **72B**: 487-491.
61. Ha, Y. C., and P. J. Barter. 1982. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. *Comp. Biochem. Physiol.* **71B**: 265-269.
62. Eisenberg, S. 1984. High density lipoprotein metabolism. *J. Lipid Res.* **25**: 1017-1058.
63. Cordle, S. R., S. J. Yeaman and R. A. Clegg. 1983. Salt-resistant (hepatic) lipase. Evidence of its presence in bovine liver and adrenal cortex. *Biochim. Biophys. Acta*. **753**: 213-219.