Density Distribution, Characterization, and Comparative Aspects of the Major Serum Lipoproteins in the Common Marmoset (*Callithrix jacchus*), a New World Primate with Potential Use in Lipoprotein Research†

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ABSTRACT: Qualitative, quantitative, and comparative aspects of the serum lipoprotein profile in the Common marmoset (*Callithrix jacchus*), a New World primate, are described. Density gradient ultracentrifugation was used to evaluate lipoprotein distribution and to establish criteria for isolation of discrete molecular fractions. The major lipoprotein classes banded isopycnically on the gradient with the following hydrated densities: VLDL, $d < 1.017$ g/mL; LDL, $d = 1.027$–1.055 g/mL; HDL fraction I, $d = 1.070$–1.127 g/mL; and HDL fraction II, $d = 1.127$–1.156 g/mL. Electrophoretic, immunological, and electron microscopic analyses attested to the purity of these fractions: the characteristics of each were assessed by chemical analysis, electron microscopy, immunological techniques, and polyacrylamide gel electrophoresis of their protein moieties. Marmoset VLDL and LDL were closely akin to those of man in size and chemical composition, although the former were richer in triglyceride; electrophoretic and immunological data showed the major protein component of VLDL and LDL to be a counterpart to human apo-B. The two HDL subfractions, i.e., HDL-I and HDL-II, corresponded in size and chemical composition to human HDL_2 and HDL_3, respectively, although slight differences in neutral lipid content were detected. By immunological and electrophoretic criteria, the major apolipoprotein of marmoset HDL was analogous to human apo-AI. In contrast, marked dissimilarities were evident in the complements of low molecular weight, tetramethylurea-soluble polypeptides of marmoset and human lipoproteins. Quantitatively, the human and marmoset lipoprotein profiles were not dissimilar, although HDL was the major class (~50%); in fasting animals, serum concentrations of VLDL, LDL, and HDL were 50–90, 170–280, and 338–408 mg/dL, respectively. *C. jacchus* was distinct from man in displaying a greater proportion of its total HDL in the less dense (HDL-II) subfraction (marmoset HDL-I/HDL-II = ~4:1; human HDL_2/HDL_3 = ~1:3). These data indicate that, as an experimental animal for lipoprotein research, the Common marmoset combines the advantages of ready availability and maintenance with a serum lipoprotein profile which resembles, in many qualitative and quantitative aspects, that found in man.

The concentrations of both LDL[1] and HDL and their relative amounts in the circulation are known to be among the factors which determine the development of human atherosclerosis (Gotto et al., 1978; Slack, 1969). Progress in understanding those aspects of metabolism which regulate serum lipoprotein levels in man may have been limited by the lack of a suitable animal species for such investigations. Thus, common laboratory animals such as the rat, guinea pig, and rabbit exhibit serum lipoprotein spectra which differ widely both qualitatively and quantitatively from that usually found in man (Calvert, 1976; Mills & Taylaur, 1971).

Although a number of studies of lipoprotein profile, structure, and metabolism have been conducted in Old World primates (Blaton & Peeters, 1976; Calvert, 1976; Kritchevsky, 1970; Mills & Taylaur, 1971; Pargaonkar et al., 1977; Rudel et al., 1977), several difficulties such as size, availability, and expense are encountered in their frequent use. Some species of New World monkeys, and in particular the squirrel (*Saimiri*) and capuchin (*Cebus*), which are more easily available, have already been employed for such studies (Hill et al., 1975; Illingworth, 1975; Portman et al., 1975; Srinivasan et al., 1976). However, to our knowledge, little effort has been made to thoroughly characterize the serum lipoproteins of one of these latter species.

In the present report, we describe our investigation of the serum lipoproteins of a New World monkey, the Common marmoset, *Callithrix jacchus*. The marmoset readily breeds in captivity and is a relatively easy and inexpensive animal to maintain (Hearn et al., 1975; Hiddleston, 1978; Ingram, 1975). Moreover, these animals can be handled and bled without difficulty.

The studies outlined herein are intended to form the basis for investigation of lipoprotein metabolism in *C. jacchus* and for the subsequent evaluation of the effects of experimental agents upon its lipoprotein profile.

Materials and Methods

Materials

Animals and Diets. The marmosets used in this study were obtained from a breeding colony which has been established at ICI Pharmaceuticals Division (Hiddleston, 1978). Most of the studies described here were made on blood samples taken from animals within the breeding facility. Pools of serum were prepared from male and female animals aged between 11 and 64 months; these were used either for the characterization of lipoproteins or for the preparation of fractions for immunization. In other instances, when animals were studied on an

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[1] Abbreviations used: VLDL, very low density lipoproteins of $d < 1.007$ g/mL unless otherwise defined; LDL, low-density lipoproteins of density as defined; HDL, high-density lipoproteins of density as defined; apo, apolipoprotein; apo-B, apolipoprotein B; EDTA, ethylenediaminetetraacetic acid; HPL, heparin-precipitable lipoproteins.

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individual basis, they were chosen to fall into closely defined age and weight ranges, as detailed in the next section. One study was made of animals maintained in a second unit where the total food intake differed significantly from that in the breeding colony.

In both units, the marmosets were caged in pairs. The temperature was maintained between 19 and 23 °C and the relative humidity between 60 and 70%.

The animals were offered a mixed diet consisting of a pelleted preparation (Mazuri Primate Diet, B. P. Nutrition, U.K.) containing the following proportions by weight of the major constituents: oil, 4.6%; protein, 21.0%; fiber, 1.6%; and carbohydrate, 42.4%. In addition, a quantity of fruit was given which consisted of apples, oranges, and bananas. Portions of malted bread ("Soreen Malt Loaf", Imperial Bakeries, Manchester, U.K.) were also offered daily. Supplements of two vitamin and mineral preparations were given along with the food ("BeMax Natural Wheat Germ", The Vitamins Co., Brentford, Middx., U.K.). Food was introduced at ~2 p.m. daily.

Blood Specimens. Blood samples were routinely taken from restrained, unanesthetized animals which had been fasted overnight for 16–18 h. Blood (up to 1 mL) was taken directly from the femoral vein through a 25 gauge needle. The period of restraint and associated stress to the animal was about 2 to 3 min. Samples were taken between 9.30 and 12.00 a.m. Serum samples were stored or transported at 4 °C, and analysis or isolation of lipoproteins commenced as soon as possible.

Other Materials. Heparin (sodium) (mucous) was supplied in a freeze-dried form (150 units/mg) by Evans Medical Ltd. (Speke, Liverpool, U.K.) and in solution as mucous injection B.P. (25 000 units/mL) by Weddel Pharmaceuticals Ltd. (London E.C.I., U.K.). Agarose of standard low molecular weight was supplied as "electrophoresis purity reagent" by Bio-Rad (Richmond, CA).

Methods

The methods used in this study are divided into three sections corresponding to different phases of the investigation. These are the following: (I) qualitative and quantitative techniques applied to pooled serum samples and designed to provide a thorough characterization of the serum lipoprotein spectrum; (II) semimicroquantitative techniques which were applied to the small volumes (~300 μL) of serum available from individual animals on a serial sampling basis; and (III) comparative studies on the immunological resemblance between human and marmoset lipoproteins.

I. (A) Detection of the Major Lipoprotein Classes of Whole Serum. Two approaches were initially used to qualitatively examine the lipoprotein content of whole marmoset serum, i.e., (1) electrophoresis on polyacrylamide gel slabs and (2) immunoelectrophoresis employing appropriate antisera.

(1) Electrophoresis. Samples (2.5 μL) of whole serum were first precolored with Sudan Black and subsequently electrophoresed on polyacrylamide gel sheets ("Lipofilm", Sebia, Issy-les-Moulineaux, France) for 1 h at 250 V and 15 mA. These sheets were constructed so as to give a discontinuous gradient from 2% monomer (origin) to 3% in the running gel. Samples of normolipidemic human serum were run alongside for comparative purposes.

(2) Immunoelectrophoresis. Marmoset whole serum was subjected to immunoelectrophoresis against its homologous antiserum in 1% agarose gel in 0.025 M veronal buffer at pH 8.6 as outlined by Scheidegger (1955). After the electrophoresis and washing and drying of the slides, they were stained for lipid with Sudan Black. For the preparation of antisera, see under Characterization of Lipoproteins—Immunological Methods.

(B) Density Gradient Centrifugation of Marmoset Serum Lipoproteins. In the first instance, density gradient centrifugation was employed to evaluate the density distribution of the lipoproteins. Application of electrophoretic, immunological, and electron microscopic techniques to the gradient fractions allowed us to define the density limits between which the major lipoprotein classes could be found. In the second application of this technique, such information was used in the preparative isolation of these lipoproteins.

Discontinuous salt gradients were constructed in cellulose nitrate tubes ($^9/_{16}$ in. diameter × 3.5 in. length). After adjustment of the nonprotein solvent density of the serum to 1.210 g/mL with solid KBr (0.325 g/mL of serum) (Hatch & Lees, 1968), five solutions were pumped successively into each tube from a Buchler Auto-Densiflow II (Searle Analytic Inc., Fort Lee, NJ) coupled to a Gilson Minipuls II peristaltic pump at a speed of 1 mL/min. These solutions were (1) 2 mL of a NaCl–KBr mixture of d = 1.240 g/mL; (2) 3 mL of serum adjusted to d = 1.21 g/mL; (3) 2 mL of NaCl–KBr solution of d = 1.063 g/mL; (4) 2.5 mL of NaCl–KBr solution of d = 1.019 g/mL; and (5) 3 mL of NaCl of d = 1.006 g/mL. Control gradients were also constructed, and these contained 3 mL of NaCl–KBr solution of d = 1.21 g/mL instead of the adjusted serum sample. All salt solutions contained 0.1% sodium azide, 0.04% EDTA, and 0.005% merthiolate, and their densities were established with a precision digital density meter (Anton Paar, Graz, Austria, Model DMA 40) at 15 °C.

The gradients were carefully placed in the SW-41 Ti rotor and centrifuged for 48 h at 40 000 rpm and at 15 °C in the Beckman L5-50 ultracentrifuge. No braking was employed at the end of the run.

In the first series of experiments, the serum-containing gradients were divided into 12 approximately equal fractions by stepwise aspiration with a narrow bore Pasteur pipet; in each case, the volume removed was adjusted to correspond so far as possible to either visible bands of lipoproteins or apparently lipoprotein-poor zones. The background density of each fraction was determined by reference to the density profile obtained from control gradients.

Fractions thus collected from the serum-containing gradients were dialyzed exhaustively at 4 °C in Spectrapor membrane tubing (molecular weight cutoff 3500; Spectrum Medical Industries, Los Angeles, CA) against a solution containing 0.05 M NaCl, 5 mM Tris-HCl, 0.04% EDTA, 0.02% sodium azide, and 0.005% merthiolate at pH 7.4.

For the preparative isolation of the lipoproteins from the second series of gradients, fractions were removed as follows: (1) VLDL, seen as a lactescent layer, was taken off in the top 1 mL of the gradient, i.e., of d < 1.017 g/mL; (2) LDL was clearly resolved as a whitish band and removed in a 1.6-mL volume corresponding to d = 1.027–1.055 g/mL; (3) the major portion of HDL, denoted as HDL-I, was seen as a faintly yellowish band and removed in a volume of 1.2 mL corresponding to d = 1.070–1.127 g/mL; (4) a second HDL fraction, which contained significant amounts of lipoproteins and was denoted as HDL-II, was also collected (in a volume of 1.7 mL) and corresponded to the density range 1.127–1.156 g/mL. The major lipoprotein fractions, interlipoprotein regions, and bottom fractions were all dialyzed and retained for study by the methods detailed below.

(C) Characterization of Lipoproteins. (1) Chemical Analyses. The lipid and protein moieties of each lipoprotein fraction

(0.5–5 mg) were first separated by extraction in organic solvents (Brown et al., 1969; Chapman et al., 1975) at 4 °C. Silicic acid column chromatography was then used to fractionate the various lipid components, the procedure employed being essentially that of Hirsch & Ahrens (1958). Fractionations were performed on duplicate samples. Individual lipid classes were then assayed as follows. Cholesteryl esters were determined in essentially the same manner as the unesterified cholesterol, i.e., by the Liebermann–Burchard reaction (Abell et al., 1952), but after hydrolysis to the free sterol; the original amount of cholesteryl ester was calculated as 1.67× that of ester cholesterol, this factor being the ratio of the average molecular weight of cholesteryl ester to free cholesterol (the average molecular weight of the fatty acids of human lipoprotein cholesteryl esters was thus used to estimate the molecular weight of marmoset cholesteryl esters, and this constitutes an assumption). Triglycerides were determined by the method of Biggs et al. (1975), using triolein as the calibration standard. The total phospholipids were assayed for phosphorus by Bartlett's procedure (Bartlett, 1959), and the original quantity of phospholipid was estimated as the phospholipid phosphorus content multiplied by 25.

The precipitated protein was solubilized, after washing, in either 1 N NaOH or a solution (denoted as solution S) containing 1% (w/v) sodium dodecyl sulfate, 1% β-mercaptoethanol, and 0.01 M sodium phosphate at pH 8.0 and assayed for protein by Lowry's method (Lowry et al., 1951).

Controls for the presence of sodium dodecyl sulfate and β-mercaptoethanol were included as appropriate; bovine serum albumin (Sigma) was used as the working standard.

(*2*) *Electron Microscopy.* Samples of each fraction (protein concentration 0.05–0.2 mg/mL) were negatively stained with 2% potassium phosphotungstate (Merck) as described earlier (Chapman & Goldstein, 1976) and essentially according to Forte et al. (1968). Stained preparations, generally made in triplicate, were examined at 60 kV with Philips EM 300 electron microscope equipped with an anticontamination device and an objective aperture of 30-μm diameter and whose magnification had been precalibrated. Micrographs of at least two grids were taken and the negatives employed for the determination of particle diameters; normally a minimum of between 100 and 200 particles was measured on each of up to five negatives. Such particles were apparently intact and freely dispersed. Sizes were determined with a microcomparator (L'Optique Scientifique, Paris).

(*3*) *Immunological Methods.* The lipoprotein fractions employed as immunogens were isolated by sequential flotational ultracentrifugation essentially according to Havel et al. (1955) under conditions previously described (Chapman et al., 1977); the density limits were selected on the basis of the density distribution profile determined above.

Antisera to marmoset whole serum, to marmoset LDL (d = 1.020–1.050 g/mL), and to marmoset HDL (d = 1.080–1.200 g/mL) were prepared in rabbits as previously described (Chapman & Goldstein, 1976) by using as immunogens 0.3 mL of whole serum, 500 μg of LDL protein, and 750 μg of HDL protein, respectively.

Double immunodiffusion and immunoelectrophoresis were carried out by the techniques of Ouchterlony (1964) and Scheidegger (1955), respectively.

(*4*) *Analysis of the Protein Moiety.* The protein moieties of each lipoprotein fraction recovered by centrifugation after solvent extraction (Brown et al., 1969; Chapman et al., 1975) and solubilization in detergent solution (solution S) were examined by electrophoresis in sodium dodecyl sulfate (NaDodSO$_4$)–polyacrylamide disc gel. The method employed was that of Weber & Osborn (1969); 3.3, 7.5, and 10% acrylamide concentrations were used. All samples (~100 μg of protein), including molecular weight standards, were pretreated by incubation at 90 °C for 3 min after solubilization. Protein fixation and staining with Coomassie Brilliant Blue R (Sigma) have been previously outlined (Chapman et al., 1975). For determination of molecular weights, a calibration curve was constructed from two series of polymerized molecular weight markers (BDH Biochemicals Ltd., Poole, U.K.), ranging from 14 300 to 71 500 and from 53 000 to 265 000.

The complement of low molecular weight, tetramethylurea-soluble apolipoproteins in the protein moiety of each lipoprotein fraction was evaluated by electrophoresis in the alkaline polyacrylamide disc gel system of Davis (1964), as modified by Kane (1973). About 100 μg of tetramethylurea-soluble protein was applied to each gel (7.5% monomer containing 8 M urea at pH 8.91); samples were normally run in duplicate in the Buchler system (Searle Analytic Inc.) at 2.5 mA/tube with water cooling. Tetramethylurea was supplied by Merck–Schuchardt and was 99% pure by gas–liquid chromatography. Gels were fixed, stained with Coomassie Brilliant Blue R, and destained as detailed elsewhere (Chapman et al., 1975). Densitometric scanning of gels was performed with a modified Gilford 250 spectrophotometer at 550 nm; densitometric areas were determined gravimetrically on peaks cut from the scan.

II. (*A*) *Serum Lipid Concentrations.* Determinations of the total serum concentrations of triglyceride and of cholesterol were made according to the procedures of Biggs et al. (1975) and of Roeschlau et al. (1974), respectively; the enzymatic kit supplied by Boehringer/Mannheim GmbH was utilized in the latter assay. Standards for calibration purposes were purified cholesterol (98.5%, Merck) in ethanol solution and triolein (99%, Sigma). In the enzymatic assay, EDTA was added to the enzyme color reagent to give a final concentration of 10 mM. In the Gilford 3500 analyses, 1 mL of this reagent was added to lipoprotein fractions or to 10 μL of whole serum; the optical density at 510 nM was measured after incubation for 25 min at room temperature.

(*B*) *HDL Cholesterol Estimation after Heparin–Mn^{2+} Precipitation.* An aliquot of 100 μL of serum was mixed with 10 μL of heparin (Evans) solution and 10 μL of $MnCl_2$ to give final concentrations of 182 IU/mL heparin and 90 mM $MnCl_2$ (Warnick & Albers, 1978).

Precipitation was allowed to continue at 4 °C overnight before centrifuging at 500g for 20 min at 4 °C. Two 30-μL portions of the clear supernatants were used for cholesterol determination. In a series of samples from six male and six female animals, the supernatants thus obtained were examined for complete precipitation of VLDL and LDL by using the anti-LDL employed in radial immunodiffusion and immunoelectrophoresis. In only one instance was any immunoreacting material detected, but this amounted to less than 1% of the total serum apo-LDL.

(*C*) *Heparin-Precipitable Lipoproteins* (*HPL*). This was a nephelometric measurement based essentially on the method of Stone et al. (1976). Thirty microliters of serum was added to 4 mL of 25 mM $CaCl_2$, and the increase in light scattering intensity (LSI) due to the addition of heparin (Weddel) to a final concentration of 30 IU/mL was measured. The increase in LSI was taken as the HPL concentration (units) of the serum. The coefficient of variation for this procedure was 6% (n = 9).

(*D*) *Measurement of Apo-LDL.* Radial immunodiffusion was performed essentially according to the method of Mancini et al. (1965), utilizing the anti-LDL antiserum at a concentration of 0.5% in the gel. Samples and standards were dispensed in 5-μL aliquots into 3-mm diameter wells. Serum samples were diluted 1:10 before use, and standards were dilutions of a marmoset serum in which the protein content of the LDL fraction (1.019–1.063 g/mL) had previously been determined after preparative ultracentrifugation.

(*E*) *Determination of Total Low-Density (VLDL + LDL) and High-Density (HDL) Lipoprotein Cholesterol.* The lipoprotein cholesterol content of the total low-density and high-density substances ($d < 1.063$ g/mL and $d > 1.063$ g/mL, respectively) was determined as follows. An aliquot of 100 μL of serum was diluted with 0.15 M NaCl and adjusted to a density of 1.063 g/mL by the addition of a suitable NaBr density solution. Two 175-μL aliquots of this mixture were centrifuged at 130000*g* for 2 h at room temperature in a Beckman airfuge. The tubes were divided into two portions in a tube slicer. The contents of the top and bottom fractions were quantitatively removed, washing each half of the tube with a further amount of 0.15 M NaCl. The cholesterol content was determined as outlined above. Coefficients of variation were determined for this procedure and were 3.3 and 5.0% (nine replicate analyses on different occasions) for the $d < 1.063$ g/mL and $d > 1.063$ g/mL fractions, respectively. Preliminary experiments showed that 2 h of centrifugation at 130000*g* was sufficient to cause virtual equilibration of the lipoproteins in the microcentrifuge tube. Some 95% of HPL and 89% of apo-LDL were recovered in the top fraction. Continuation of centrifugation for up to 6 h did not cause any significant alteration in recoveries of cholesterol, HPL, and apo-LDL in the top and bottom fractions.

III. Comparative Studies. For further evaluation of the relationship between marmoset and human serum lipoproteins, several immunological procedures were employed by using antisera to human whole serum and to human lipoproteins. Horse antiserum to human whole serum was purchased from the Institut Pasteur (Paris), while rabbit antiserum to human serum LDL (d = 1.024–1.045 g/mL) and HDL (d = 1.090–1.190 g/mL) and human apolipoprotein B were prepared as outlined elsewhere (Chapman & Goldstein, 1976). The amounts of protein antigen injected were 300 μg, 1 mg, and 500 mg, respectively. Human apo-B was prepared from detergent-solubilized apo-LDL by gel filtration chromatography (Herbert et al., 1973; Chapman et al., 1977). Antiserum to human serum Lp (a) was a gift from Dr. G. Kostner.

Results

Identification of Lipoproteins in Whole Serum. Immunodiffusion and immunoelectrophoresis of marmoset whole serum against its homologous antiserum revealed the presence of two major lipoprotein classes, which presented precipitin lines of β and α electrophoretic mobility, respectively (Figure 1; immunodiffusion pattern not shown). It is noteworthy that the precipitin line due to γ-globulins stained faintly with Sudan Black, thus indicating the presence of small amounts of lipid (possibly fatty acids).

Electrophoresis of whole serum samples on polyacrylamide gel sheets typically revealed three strongly staining bands (Figure 2). One of these was situated at the junction of the 2 and 3% gels and corresponded to the position of VLDL in the human pattern (Figure 2B) and a second was located a short distance into the 3% gel in a position which closely resembled that of LDL in human serum, while a third had migrated substantially further into the 3% gel and was similar

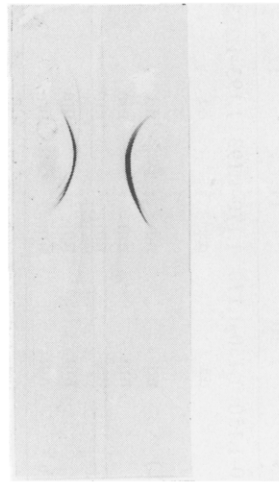

FIGURE 1: Detection of the two principal marmoset lipoprotein classes by immunoelectrophoresis. Two different marmoset whole sera (20 μL) were reacted with the homologous antiserum (central trough, 100 μL), and the slide was stained for lipid with Sudan Black.

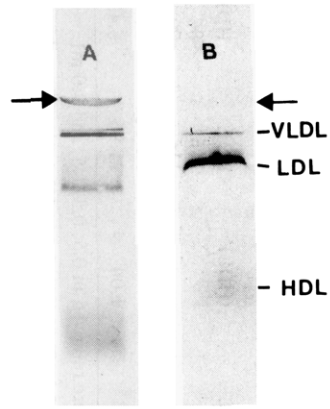

FIGURE 2: Electrophoresis of marmoset and human whole serum on discontinuous polyacrylamide gel sheets. Serum samples were prestained with Sudan Black: (A) marmoset; (B) human. Arrows indicate the point of sample application.

to human HDL in its migration. It is noteworthy that on occasion (~30% of the sera) the LDL band was preceded by a diffuse area at the beginning of which there was a faint band (denoted as LDL[1]); Lp (a) of human serum migrates to this region. It should also be mentioned that the HDL band was occasionally resolved into two bands.

Density Distribution of Marmoset Serum Lipoproteins. In the first series of density gradients, fractionated for the determination of lipoprotein density distribution, a series of 12 successive fractions of ~1 mL were obtained; the immunological reactivity, the electrophoretic mobility in polyacrylamide gel, and the particle size distribution of these fractions were subsequently evaluated and are summarized in Table I.

Fractions 1–5, corresponding to a density range of 1.006–1.054 g/mL, displayed β reactivity upon immunoelectrophoresis against an antiserum to marmoset whole serum; the strongest reactivity was detected in fractions 1 ($d < 1.017$ g/mL) and 3–5 (d = 1.023–1.054 g/mL). Fraction 6, d = 1.054–1.061 g/mL, also contained trace amounts of β-reacting material. Lipoproteins with α reactivity were identified essentially in fractions 6–8 (d = 1.054–1.120 g/mL) and in trace amounts in fraction 5 and in fractions 9–11 (d = 1.120–1.25 g/mL). Albumin and other serum proteins were present in fractions 11 and 12, i.e., at $d > 1.170$ g/mL.

Electrophoresis in polyacrylamide gel slabs confirmed the above results, revealing lipoproteins with the mobility of VLDL

Table I: Evaluation of the Density Distribution of Marmoset Serum Lipoproteins

	density gradient fraction no.[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
density limits (g/mL)[b]	<1.017	1.017–1.023	1.023–1.027	1.027–1.038	1.038–1.054	1.054–1.061	1.061–1.095	1.095–1.120	1.120–1.150	1.150–1.170	1.170–1.193	1.193–1.253
immunological reactivity[c]	β++	β+	β++	β++	β++	β±	α+	α±	α±	α±	α±	α±
					α±	α+						
electrophoretic mobility[d]	VLDL	nd	LDL	LDL	LDL	(HDL)	HDL	HDL	(HDL)	nd	nd	nd
particle sizes (A):[e] mean	328	nm	217	205	197		116	86	nm	nm	nm	nm
mode	270	nm	216	196	185		115	90	nm	nm	nm	nm
range	200–630	nm	180–250	160–220	160–200		92–160	54–110	nm	nm	nm	nm
lipoprotein protein content (mg)	1.51	0.35	0.47	4.96	2.03	1.04	21.96	6.43	4.56	nm	nm	nm

[a] Fractions were of ~1-mL volume, with the exception of fractions 3, 6, 7, and 12, which were removed in 0.5, 0.5, 1.5, and 2.0 mL, respectively (see Density Distribution of Marmoset Serum Lipoproteins). [b] Density limits were taken from a standard curve of density vs. volume derived from control gradients containing only salt solutions. [c] Immunological reactivity was assessed by immunoelectrophoresis using antiserum to marmoset whole serum. The degree of reactivity was estimated on the basis of the strength of lipid staining of the precipitin line: (++) strong reactivity, (+) moderate reactivity, and (±) weak reactivity. [d] Electrophoretic mobility in polyacrylamide gel slabs was assessed by comparison with the lipoproteins of human serum; parentheses indicate the presence of small but detectable amounts. nd, not detectable. [e] Particle sizes, in angstroms, were determined from electron micrographs of negatively stained lipoproteins (see Methods). nm, not measured.

in fraction 1 and of LDL in fractions 3–5 (Table I). Lipoproteins with the mobility of HDL were clearly identified in fractions 6–9.

Examination of the particle sizes of each gradient fraction showed a decrease in diameter concomitant with an increase in density. Thus, particles with hydrated densities less than 1.017 g/mL, in this case VLDL, exhibited a wide range of diameter (200–630 Å) and mean of 328 Å, while LDL, with hydrated densities in the ranges 1.023–1.027, 1.027–1.038, and 1.038–1.054 g/mL, displayed mean diameters of 217, 205, and 197 Å, respectively; HDL, with hydrated densities in the ranges 1.061–1.095 and 1.095–1.120 g/mL, were still smaller, having average diameters of 116 and 86 Å, respectively.

The total protein contents of the respective fractions reveal peaks at densities of less than 1.017, 1.027–1.038, and 1.061–1.095 g/mL, corresponding to VLDL and the principle species of LDL and HDL, respectively; particularly low levels of protein were seen in fractions 2, 3, and 6 of densities 1.017–1.023, 1.023–1.027, and 1.054–1.061 g/mL, respectively.

Characterization of Serum Lipoproteins. The results presented above indicated that the major species of LDL and HDL in the marmoset were essentially located within the density ranges of 1.027–1.054 and 1.061–1.120 g/mL, respectively. This information was used to decide the density ranges for the preparation of marmoset lipoproteins by density gradient centrifugation, which were as follows: VLDL, $d < 1.017$ g/mL;[2] LDL, $d = 1.027$–1.055 g/mL; HDL fraction I, 1.070–1.127 g/mL; HDL fraction II, 1.127–1.156 g/mL. These fractions contained respectively 2.3, 24.2, 48.9, and 14.9% of the total lipoprotein protein recovered from the gradient.

Chemical Composition. The mean weight percent chemical composition of marmoset lipoproteins is presented in Table II and compared with the corresponding fractions from human serum.

The marmoset fraction of $d < 1.017$ g/mL displayed a composition which clearly resembled that of the corresponding fraction from human serum isolated by the same procedure, although the ratio of cholesteryl ester/free cholesterol was rather high in the former (4.3:1 and 2.5:1, respectively).

Marmoset LDL resembled its human counterpart with the exception of its almost twofold higher triglyceride content; the proportion of this neutral lipid may, however, vary considerably in different persons (Lee, 1976).

The total marmoset HDL fraction, with a hydrated density in the range 1.061–1.150 g/mL and corresponding to the major gradient fractions in which HDL α reactivity was detected (see Table I), was similar to that of human HDL isolated in the interval $d = 1.063$–1.21 g/mL. However, it contained a somewhat higher proportion of cholesteryl ester than the equivalent human fraction. Moreover, the total contribution of neutral lipids to the weight of marmoset HDL (27.4%) was more than that of man (23.0%) while that of its protein moiety was rather less (46.2% as compared to 51.9% in man), observations suggestive of a relatively low peak hydrated density for the monkey fraction. This finding is not inconsistent with the data in Table I since the major HDL particles were distributed within the (hydrated) density limits 1.061–1.095 g/mL.

The major HDL subfraction in the marmoset (HDL-I) accounted for 76% of the total HDL on a protein weight basis and displayed an elevated ratio of esterified to free cholesterol

[2] It is noteworthy that the present density gradient procedure does not allow complete removal of the VLDL fraction in a volume corresponding to $d < 1.007$ g/mL.

Table II: Mean Percent Weight Chemical Composition of Marmoset Serum Lipoproteins

	mean % wt composition							
	VLDL		LDL		marmoset		HDL	
	marmoset	man	marmoset	man	HDL-I	HDL-II	marmoset	man
density (g/mL)	<1.017	<1.017	1.027–1.055	1.027–1.053	1.070–1.127	1.127–1.156	1.061–1.150	1.063–1.21
no. of preparations	4[a]		4		2	2	2	
cholesteryl ester	16.1 ± 3.8	16.6[b]	37.4 ± 2.2	38.4[b]	21.3	14.2	21.3	15.0[c]
free cholesterol	3.7 ± 1.6	6.7	7.3 ± 2.6	10.0	2.2	3.8	2.2	2.9
triglyceride	57.3 ± 6.8	56.5	10.9 ± 2.0	6.5	8.7	6.0	6.1	8.0
phospholipid	14.9 ± 1.8	13.1	20.9 ± 1.6	22.4	27.9	17.3	24.2	22.7
protein	8.1 ± 1.6	9.5	23.5 ± 2.2	22.1	39.8	58.7	46.2	51.9

[a] Values are the means ± SD of duplicate analyses of the number of preparations. [b] Data from M. J. Chapman, S. Goldstein, D. Lagrange, I. Tayeb, and M. H. Laudat (unpublished experiments); fraction was isolated by gradient ultracentrifugation. [c] Data from Mills & Taylaur (1971); fraction was isolated by flotational ultracentrifugation.

(CEFC = 9.7:1). In its composition, it resembles the band III subfraction isolated by the same technique from human serum by M. J. Chapman, S. Goldstein, D. Lagrange, I. Tayeb, and M. H. Laudat (unpublished experiments), which is similar to the HDL_2 subclass described by others (Mendoza et al., 1976). The minor subfraction of marmoset HDL, fraction II, is akin to human HDL_3 as isolated in the density range 1.125–1.200 g/mL by Anderson et al. (1978), except that it contains a higher proportion of triglyceride (6.0 and 1.6%, respectively).

Electron Microscopic Studies. Electron microscopic examination of the negatively stained particles in the VLDL fraction ($d < 1.017$ g/mL) revealed essentially spherical particles which readily deformed to give polygonal shapes on contact (Figure 3A). In the representative preparation shown, their diameter ranged from about 235 to 450 Å; occasional particles with diameters of the order of 550 Å were noted, but these accounted for ≤5% of the total. In a representative preparation, the mean diameter was 318 Å and the modal diameter was 270 Å. These results concur with those in Table I for a different preparation with the same density. It is noteworthy that in the particle size distribution shown in Figure 3A two principal populations of VLDL particles, 270 and 360 Å in size, occur; this was not a consistent observation.

In a representative LDL (d = 1.027–1.055 g/mL) preparation, the particles presented an essentially spherical shape and finely granulated surface; no subunit structure was discernible, and little deformation occurred in particles in contact (Figure 3B). Their mean diameter was 210 Å; almost 75% of the particles were in the size range of 180–216 Å. The modal diameter in the fraction shown was 216 Å, and the overall range was 180–270 Å; less than 5% of the particles were larger than 250 Å.

The intermediate fraction between LDL and HDL-I of d = 1.055–1.070 g/mL was rather heterogeneous, containing particles ranging in size from 130 to 234 Å. Their mean diameter was 165 Å. In contrast, the HDL-I fraction (d = 1.070–1.127 g/mL) immediately beneath it was rather homogeneous; the mean particle diameter was 100 Å (Figure 3C). The modal diameter varied and was either 90 or 108 Å according to the preparation. The overall range in size was narrow and typically 90–126 Å (Figure 3C). Particles were approximately spherical but were occasionally polygonal in crowded fields.

The particles in the HDL-II fraction (d = 1.127–1.156 g/mL) constituted a distinct population from that in HDL-I. Thus, the major particle present (>80% of the total) was of 71-Å diameter, with small amounts of 57-Å HDL (Figure 3D); the mean diameter was 69 Å. Only 3% of the particles (of diameter 85 Å) were present with size superior to 71 Å. These lipoproteins were thus highly homogeneous in size. They typically presented a spherical profile and apparently lacked subunit structure (Figure 3D).

With regard to the purity and degree of overlap of the four lipoprotein fractions, our electron microscope studies indicate a small contamination of LDL with VLDL (overall size ranges of 180–270 and 235–450 Å, respectively), although this would appear to amount to only ~5% of the total particle populations in each case; such an overlap may be explained by the small proportion of d = 1.006–1.017 g/mL particles removed in the gradient VLDL fraction. Alternatively, particles with sizes approaching those of VLDL, but with density and composition resembling those of LDL, could be present in the density interval 1.027–1.055 g/mL.

The particle size distributions of the LDL, HDL-I, and HDL-II fractions (180–270, 90–126, and 57–85 Å, respectively) clearly indicate that each represents a discrete lipoprotein (size) population, with essentially no overlap.

The particle sizes of VLDL ($d < 1.006$ g/mL) and LDL (d = 1.020–1.050 g/mL) isolated by sequential preparative ultracentrifugation concorded well with those found for the corresponding lipoproteins separated on the density gradient. Thus, in two different VLDL samples, the mean diameters were 443 and 484 Å and the ranges were 240–600 and 230–690 Å, respectively; in the LDL fractions from the same sera, the mean sizes were 217 and 200 Å and the ranges were 180–270 and 180–252 Å, respectively.

Immunological Studies. Upon examination by immunodiffusion and immunoelectrophoresis, marmoset VLDL and LDL reacted with antiserum to marmoset whole serum and to marmoset LDL to produce single precipitin lines which joined in a reaction of identity with each other and with the corresponding line formed against marmoset whole serum; this line was characteristic of β-reacting material (Figure 4). In the reaction of VLDL with these antisera, the precipitin line was faint and poorly stained, probably as a result of the small amount of VLDL used. In contrast to the reactivity of VLDL and LDL, the HDL-I and -II fractions exhibited weak but distinct α reactivity under similar conditions (not shown).

Analysis of the Protein Moieties. The totally delipidated protein moieties of VLDL and LDL appeared only partially soluble in aqueous buffers lacking detergents; in the presence of a suitable detergent, such as sodium dodecyl sulfate in phosphate buffer (i.e., solution S), complete solubility was obtained.

Examination of the detergent-solubilized (total) apoproteins of VLDL, LDL, and HDL fractions by electrophoresis in $NaDodSO_4$–polyacrylamide gel (3.3% monomer) showed the

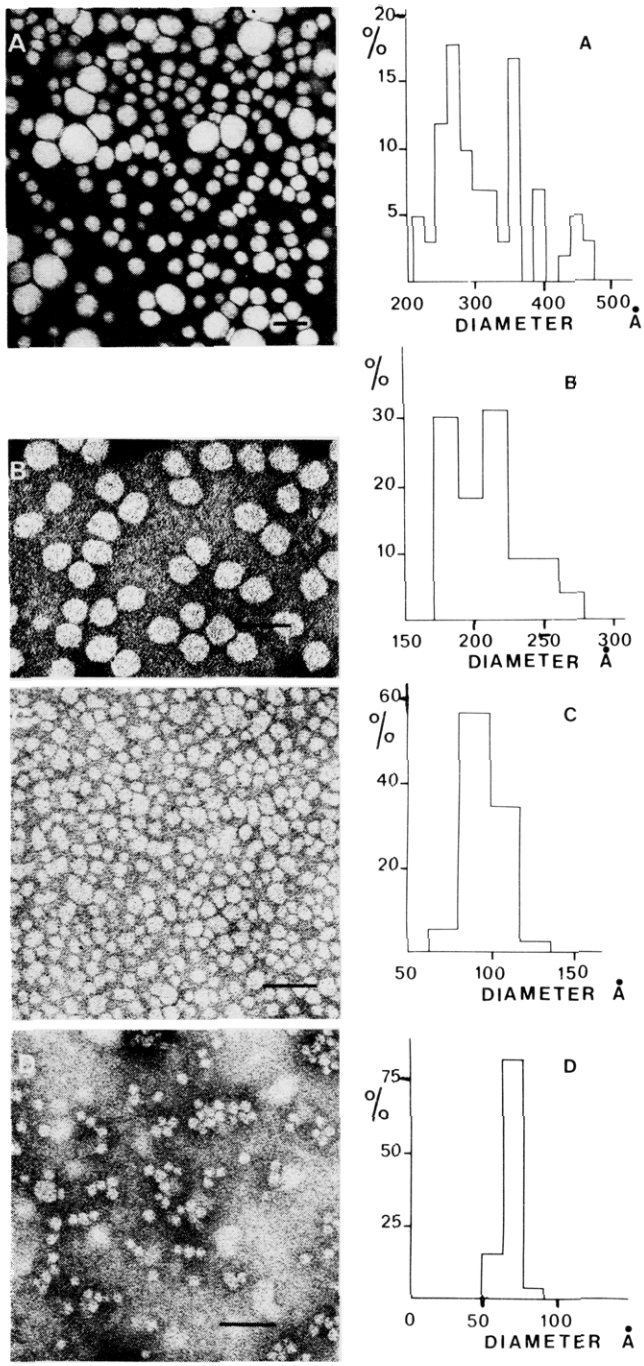

FIGURE 3: Electron micrographs of negatively stained marmoset serum lipoproteins isolated by density gradient centrifugation and the frequency distribution of particle diameters in each fraction. Samples are (A) VLDL of $d < 1.017$ g/mL, (B) LDL of $d = 1.027$–1.055 g/mL, (C) HDL-I of $d = 1.070$–1.127 g/mL, and (D) HDL-II of $d = 1.127$–1.156 g/mL. At the left is shown a representative electron photomicrograph of each fraction, the bars representing 500 Å. At the right is the corresponding frequency distribution of particle diameters, from which up to 5% of the occasional particles detected at either extreme of the ranges have been excluded.

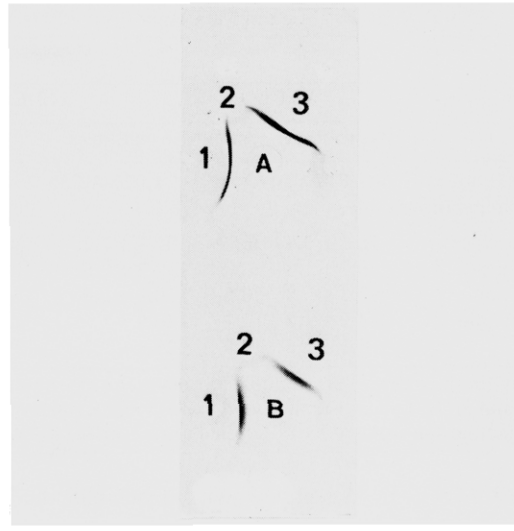

FIGURE 4: Immunodiffusion pattern of marmoset serum and lipoprotein fractions (lipid staining). Central wells: (A) antiserum to marmoset whole serum (25 μL); (B) antiserum to marmoset LDL (25 μL). Peripheral wells: (1) marmoset whole serum (20 μL); (2) marmoset VLDL (≃10 μg); (3) marmoset LDL (15 μg).

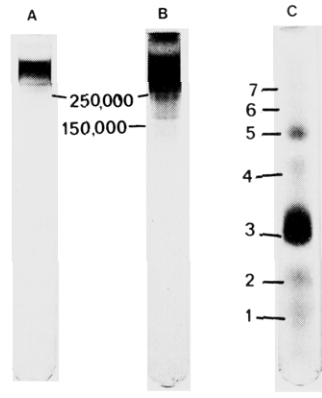

FIGURE 5: Electrophoretic patterns in $NaDodSO_4$–polyacrylamide gel of the total apoproteins of the principal classes of marmoset serum lipoproteins isolated by density gradient centrifugation. Samples of (A) apo-VLDL ($d < 1.017$ g/mL) and (B) apo-LDL ($d = 1.027$–1.055 g/mL) were electrophoresed in 3.3% monomer gels; a sample of (C) apo-HDL ($d = 1.061$–1.150 g/mL) was run in a 7.5% gel. Gels were stained with Coomassie Brilliant Blue and molecular weights calculated from a series of purified, polymerized marker proteins. The molecular weights of numbered bands are given in the text.

major component of both apo-VLDL and apo-LDL to be present as an intensely staining broad band whose low migration corresponded to a protein of high molecular weight (>250 000) (parts A and B of Figure 5). This major band was often resolved as a doublet with further minor components immediately beneath it, their molecular weights ranging from ca. 230 000 to 150 000. Such general behavior in 3.3% gels is typical of that of the major protein component of human apo-LDL, i.e., apolipoprotein B, when electrophoresed under these conditions in our laboratory (Chapman & Goldstein, 1976, Chapman et al., 1977). In addition to this apo-B-like component in marmoset apo-VLDL and apo-LDL, an additional band of variable molecular weight (75 000–85 000) was detectable in each of them. Apo-VLDL did, however, tend to be distinguished by the presence of small amounts of diffusely staining material ranging from about 5000 to 30 000 in size.

The electrophoretic pattern characterizing apo-HDL fractions (i.e., total HDL of $d = 1.061$–1.150 g/mL and HDL subfractions I and II) was quite distinct from those of apo-VLDL and apo-LDL since in 3.3% monomer gels no protein was detectable with $M_r > 90\,000$ (thus implying the absence of any apo-B-like constituent), the two major components exhibiting molecular weights of 26 000–29 000 and 14 000–18 000. In addition, small amounts of components with molecular weights of 60 000–65 000 and 80 000–85 000 were identified. Electrophoresis in gels of 7.5% monomer gave superior resolution of HDL apoproteins, resolving polypeptides with molecular weights of 9000–12 000 (1), 17 000–18 800 (2),

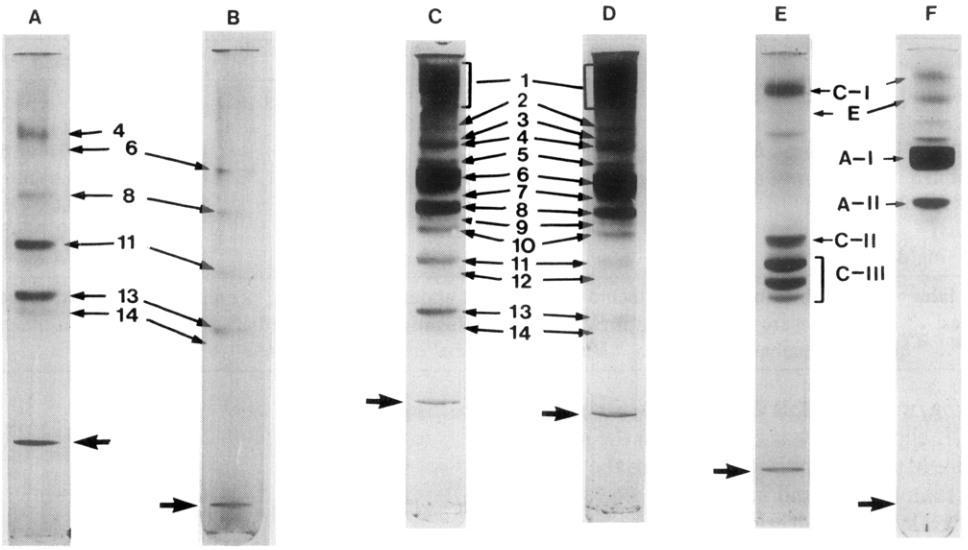

FIGURE 6: Electrophoretic patterns of tetramethylurea-soluble apoproteins of marmoset and human serum lipoproteins in basic polyacrylamide disc gel. Samples of the tetramethylurea-soluble extracts, derived from 100–150 μg of lipoprotein protein, were electrophoresed in 7.5% monomer gels containing 8% urea at pH 8.9; staining was performed with Coomassie Brilliant Blue. Both marmoset and human serum lipoproteins were prepared by density gradient centrifugation. Samples are (A) marmoset VLDL ($d < 1.017$ g/mL), (B) marmoset LDL ($d = 1.027$–1.055 g/mL), (C) marmoset HDL-I ($d = 1.070$–1.127 g/mL), (D) marmoset HDL-II ($d = 1.127$–1.156 g/mL), (E) human VLDL ($d < 1.017$ g/mL), and (F) human HDL_3 ($d = 1.095$–1.140 g/mL). Marmoset apolipoproteins are numbered in descending order; the arrows indicate the dye front in each gel. Human apolipoprotein nomenclature is according to Alaupovic (1972).

26 500–28 000 (3), 42 000–46 000 (4), 60 000–61 000 (5), 80 000–85 000 (6), and ~90 000 (7) (Figure 5C); of these, the bands denoted as (3), (5), (1), and (4) were the most intensely stained, the other bands probably representing minor components.

Electrophoresis of the tetramethylurea-soluble polypeptides of marmoset lipoproteins in 7.5% polyacrylamide gels containing urea at alkaline pH revealed up to 14 different components. Comparison of bands in different gels was facilitated by calculation of their relative electrophoretic mobilities,[3] this index being employed in their identification.

VLDL ($d < 1.017$ g/mL) was characterized by its dominant proportion of polypeptides of high mobility (bands 11–14, electrophoretic mobilities 0.45–0.66); bands 11 and 13 predominated (Figure 6A), amounting together to 50% of the total on a densitometric basis. Small amounts of components with intermediate and low mobility were also detectable (bands 8 and 4 amounting to about 15 and 25% densitometrically and with electrophoretic mobilities of 0.35–0.37 and 0.20–0.22, respectively). On occasion, traces of band 6 (mobility ~0.25) were noted. The electrophoretic pattern given by LDL qualitatively resembled that characteristic of VLDL, bands 6, 8, 11, 13, and 14 generally being present and in similar proportions; LDL could be distinguished only by its lack of band 4 and by the apparently small proportion of its protein moiety which was constituted by tetramethylurea-soluble polypeptides since application of some 150 μg of LDL protein (Figure 6B) realized the detection only of faint bands.

The electrophoretic pattern characteristic of HDL and its subfractions (parts C and D of Figure 6) was distinct from those of VLDL and LDL; its major components were bands 6 and 8, representing respectively 20–35 and 10–20% densitometrically. Band 1 was always resolved as a diffuse region in the gels; its densitometric estimation indicated it to account for 20–35% of the total, although this value may be falsely elevated. Band 4 was also a principal component since it usually accounted for between 8 and 12% of the total. The HDL pattern was unaffected by reducing agents.

Upon comparison of the patterns and densitometric scans of the HDL-I and HDL-II subfractions, it was evident that there was a lower proportion of fast-migrating apolipoproteins in the denser subfraction (i.e., HDL-II); thus, bands 11–14 together amounted to ~10% of the HDL-I-soluble components while those in HDL-II accounted for less than 5%. Furthermore, HDL-I was distinguished from HDL-II in that the ratio of the major HDL apoproteins, i.e., bands 5 and 6, was rather lower in the former (1.7:1 in HDL-I and 2.3:1 in HDL-II on a densitometric basis; such an estimate is at most semiquantitative).

Examination of the electrophoretic patterns in $NaDodSO_4$–polyacrylamide gel and in the alkaline urea system of VLDL, LDL, and HDL isolated by ultracentrifugal flotation failed to reveal any notable differences from the patterns given by the corresponding density gradient fractions. Comparison of the patterns in polyacrylamide disc gel of the soluble apolipoproteins of marmoset and man revealed that the human C-I and E components (see parts E and F of Figure 6) exhibited similar mobility to band 1 and bands 3 and 4, respectively, in the marmoset patterns. The mobilities of the major human HDL apoproteins, A-I and A-II, also resembled those of the principal marmoset HDL bands, i.e., bands 6 and 8. In contrast, the overall pattern in the region typical of the human C-II peptides was rather distinct from that in the corresponding zone of the marmoset gels.

Comparison of Lipid and Lipoprotein Cholesterol Levels in Male and Female Marmosets. In this comparison, 1-mL blood samples were obtained from nonfasting animals (12 males and 12 females) in the breeding unit. All animals were aged between 11 and 13 months, and the weight ranges were males 217–338 g and females 225–335 g. The serum was analyzed as described under Methods (II), and the results are presented in Table III. The total serum cholesterol levels averaged 139 and 135 mg/dL in male and female animals, respectively, and in both sexes a similar proportion (52 and 55%) was found in the serum fraction of density less than 1.063 g/mL. The amount of cholesterol found in the $d > 1.063$ g/mL fraction was slightly more in the case of males than in

[3] Relative electrophoretic mobility represents the ratio of the distance of migration of the individual apolipoprotein to that of the dye front.

Table III: Comparison of Lipid and Lipoprotein Levels in Male and Female Marmosets[a]

	males (n = 12)	females (n = 12)
total cholesterol (mg/dL)	139.0 ± 15.3 (116–161)	134.9 ± 17.5 (108–156)
total triglyceride (mg/dL)	168 ± 104 (83–396)	189 ± 45 (132–257)
cholesterol in $d < 1.063$ g/mL fraction[b] (mg/dL)	72.4 ± 14.9 (40–95)	74.7 ± 11.6 (56–92)
cholesterol in $d > 1.063$ g/mL fraction[b] (mg/dL)	65.1 ± 10.9 (43–79)	60.7 ± 6.6 (50–68)
HDL cholesterol[c] (mg/dL)	70.5 ± 10.9 (50–89)	64.3 ± 9.6 (49–81)
HPL[d] (units)	63.2 ± 18.1 (33–87)	67.4 ± 12.7 (43–82)
apo-LDL[e] (mg/dL)	34.4 ± 6.0 (23–43)	37.3 ± 4.1 (30–44)

[a] The results in this table were obtained by using the techniques outlined under Methods (II). Results are expressed as mean ± SD. Ranges are given in parentheses. [b] Fractions were separated by airfuge ultracentrifugation. [c] Measured after precipitation of serum VLDL and LDL with heparin and Mn^{2+}. [d] Measured nephelometrically. [e] Measured by radial immunodiffusion.

females (65 vs. 60 mg/mL), but this difference was not significant. When HDL cholesterol was estimated in the supernatants after heparin–Mn^{2+} precipitation of serum, the levels were ~12% higher than those found in the $d > 1.063$ g/mL fraction. This may reflect some overlapping of lipoproteins with the physicochemical properties of HDL into the fraction of $d < 1.063$ g/mL, as was evident from the density gradient experiments described earlier. Both the total heparin-precipitable lipoproteins estimated nephelometrically and the quantity of apo-LDL estimated by radial immunodiffusion were similar in the two sexes.

Biological Variation in Lipid and Lipoprotein Cholesterol Levels. One of the objectives of the study was to determine whether the marmoset could be of use in examining the effect of certain experimental agents on the steady-state levels of serum lipoproteins. This required some knowledge of the natural variability of these parameters in animals kept under the conditions outlined previously.

For estimation of this, 12 nonfasted, male animals housed in a separate unit were used. They were selected in a narrow weight range (277–375 g) and were aged 23 to 24 months. The animals were removed from the cages once per day and given an oral dose of 0.5 mL of water to simulate conditions of administration of an experimental agent. Blood samples of 1 mL were taken once per week for a 5 week period.

The overall mean cholesterol level (210 mg/dL) of animals in this group was notably higher than those of animals in the breeding unit documented in the previous section. This seemed to reflect an overall higher intake of food by the animals in the separate unit. However, the increased amount seemed to be equally distributed into low- and high-density lipoprotein fractions so that, as before, an average of 54% of the total cholesterol was found in the $d < 1.063$ g/mL fraction.

It is noteworthy that the concentration of each lipoprotein class in individual animals remained relatively constant during the study period. Thus, the animal with the highest concentration of cholesterol in the $d < 1.063$ g/mL fraction (range 157–192 mg/dL) was consistently the highest, whereas the animal with the lowest amount of cholesterol in this fraction also remained the lowest (range 70–84 mg/dL). Similarly, characteristic levels of HDL cholesterol were observed. The animal with the highest amount (range 131–174 mg/dL, heparin–Mn^{2+} method) contrasted with the animal having the lowest level (range 72–96 mg/dL).

As observed previously, the measurement of HDL cholesterol after heparin–Mn^{2+} precipitation gave higher levels than those in the $d > 1.063$ g/mL fraction (mean values of 117 and 101 mg/dL, respectively). However, there was a good correlation between the mean results obtained in each animal with the two methods (r = 0.90). Similarly, there were also good correlations between the mean apo-LDL concentration in each animal and the amount of cholesterol in the $d < 1.063$ g/mL fraction (r = 0.90). The latter measurement also corresponded well with that of HPL (r = 0.88). It is interesting to note, however, that there was no discernible relationship between the concentration of cholesterol in the $d < 1.063$ g/mL and $d > 1.063$ g/mL fractions. This could be taken to suggest that at least some of the factors determining the steady-state levels of the two classes of lipoproteins act independently. This should be taken together with the observations described earlier that low- and high-density lipoproteins in this animal also have completely distinct protein moieties.

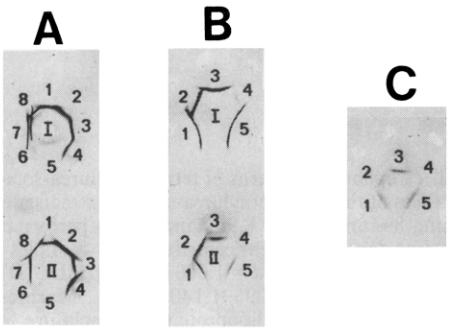

FIGURE 7: Comparative immunodiffusion patterns of human and marmoset whole serum and lipoprotein fractions. The slides were stained for lipid with Sudan Black. (A) Central wells: (I) horse antiserum to human whole serum (25 μL); (II) rabbit antiserum to marmoset whole serum (25 μL). Peripheral wells: (1) human LDL (10 μg of protein); (2) marmoset LDL (15 μg of protein); (3) human VLDL (20 μg of protein); (4) marmoset VLDL (25 μg of protein); (5) human HDL (20 μg of protein); (6) marmoset HDL (30 μg of protein); (7) human whole serum (20 μg); (8) marmoset whole serum (20 μg). (B) Central wells: (I) rabbit antiserum to human LDL (25 μL); (II) rabbit antiserum to marmoset LDL (25 μL). Peripheral wells: (1 and 5) human LDL (10 μg of protein); (2) marmoset LDL (15 μg of protein); (3) human VLDL (20 μg of protein); (4) marmoset VLDL (10 μg of protein). (C) The central well contained antiserum to human apolipoprotein B (25 μL). Peripheral wells 1–5 were as in (B) above.

Comparative Studies. Marmoset whole serum, VLDL, LDL, and HDL were compared with their human counterparts by immunodiffusion and immunoelectrophoresis, employing the respective homologous and heterologous antisera.

Upon immunodiffusion, reactions of partial identity were revealed between the β-lipoproteins and α-lipoproteins of whole marmoset and of whole human serum in the reaction with both antiserum to human whole serum and to marmoset whole serum (Figure 7A). Similarly, partial cross-reactivity occurred between marmoset and human LDL and also between the VLDL's of the two species when they were reacted against monospecific antisera to human LDL or to marmoset LDL (Figure 7B).

These results were confirmed and extended by the use of an antiserum to human apolipoprotein B, which permitted the identification of an apolipoprotein analogous to human apo-B

in marmoset LDL and to a lesser extent in marmoset VLDL (Figure 7C). It is noteworthy that in the reaction with antiserum to human apo-B a precipitation pattern corresponding to almost complete identity was obtained between human and marmoset LDL.

Human and marmoset whole serum and HDL reacted upon immunodiffusion with a monospecific rabbit antiserum to human apolipoprotein A-I.

Finally, the presence of a counterpart to the human Lp (A) lipoprotein in the marmoset was examined with the use of an antiserum to human Lp (a); we were unable to detect any reactivity in 10 different marmoset whole sera.

Discussion

The marmoset is a New World primate of the family Callitrichidae which breeds readily in captivity and can be made available in large numbers from a suitable breeding colony (Hearn et al., 1975; Hiddleston, 1978; Ingram, 1975). Moreover, this animal is safely and easily handled and could justifiably be described as a convenient laboratory species (Hearn et al., 1975). It was with these points in mind that we undertook the determination of the circulating concentrations and characteristics of the major serum lipoproteins of this species.

Some studies of the physical and chemical properties of the serum lipoproteins of other New World primates, and especially of the family Cebidae, i.e., the squirrel monkey (genus *Saimiri*), the capuchin monkey (genus *Cebus*) and spider monkey (genus *Ateles*), have been reported (Hill et al., 1975; Hojnacki et al., 1977; Illingworth, 1975; Srinivasan et al., 1972, 1974, 1976). Of these species, the most attention has been focussed on the squirrel monkey, in which the quantitative distribution together with the structure and metabolism of the major lipoprotein classes has been investigated principally by Illingworth, Portman, and colleagues (Illingworth, 1975; Illingworth & Portman, 1972; Illingworth et al., 1974, 1975; Portman et al., 1975, 1976). Some data are also available on the concentration and composition of the lipoproteins in capuchin, spider, and *Aotus* monkeys (Hill et al., 1975; Hojnacki et al., 1977; Mills & Taylaur, 1971; Pargaonkar et al., 1977; Srinivasan et al., 1972, 1974, 1976).

In most of the above studies in which ultracentrifugation has been used to isolate lipoprotein fractions (Hill et al., 1975; Hojnacki et al., 1977; Illingworth, 1975; Illingworth & Portman, 1972; Illingworth et al., 1974, 1975; Pargaonkar et al., 1977), the investigators have made the tacit assumption that the conventional density limits or flotation rates classically employed in the investigation of human serum lipoproteins are equally applicable to the species under examination. In the present study we have therefore paid particular attention to the problem of establishing correct and precise criteria for the isolation of discrete lipoprotein classes. This was made possible by application of density gradient ultracentrifugation in combination with electron microscopic, electrophoretic, and immunological techniques (Table I).

Thus, lipoproteins with the immunological reactivity, electrophoretic mobility, particle size, and hydrated density typical of the β or low-density substances (i.e., VLDL and LDL) of human serum and a number of other animal species were present from the lowest density fraction studied up to a limiting density of 1.061 g/mL. No lipoproteins with this type of immunological reactivity (i.e., β) were detectable at higher densities. The LDL fraction which separated isopycnically in the density interval 1.054–1.061 g/mL (Table I) did, however, contain small quantities of α or high-density substances, as judged both by immunological reactivity upon immunoelectrophoresis and by migration on polyacrylamide gel sheets. On the basis of these data, LDL was subsequently prepared with hydrated density in the range 1.027–1.055 g/mL. A similar interval (i.e., d = 1.027–1.053 g/mL) was used by M. J. Chapman, S. Goldstein, D. Lagrange, I. Tayeb, and M. H. Laudat (unpublished experiments) in the preparation of human LDL by the same density gradient technique.

Some comment on the decision to remove VLDL from the density gradient as a fraction of $d < 1.017$ g/mL is warranted. As noted earlier, the difficulty in removing VLDL in a small volume, added to the steepness of the gradient near the top of the tube, made it necessary to remove VLDL in the top 1 mL, which contained a range of densities of less than 1.017 g/mL. The size distribution of particles in the $d < 1.017$ g/mL fraction was very similar to that in a fraction of $d < 1.007$ g/mL isolated by the conventional flotation procedure. There was, however, as expected, a slightly higher proportion of small particles (235–290 Å) in the former compared to the latter fraction.

The physical, chemical, and immunological properties of marmoset VLDL resembled those typical of a human fraction ($d < 1.017$ g/mL) isolated by the same density gradient techniques. There were only minor differences evident between the percent chemical compositions (Table II), but notably a comparatively low unesterified cholesterol content was found in the marmoset VLDL. The closeness of the chemical composition taken together with an identical morphology and the similar size distribution of human and marmoset VLDL (mean and ranges: 318, 235–450 Å and 317, 207–575 Å, respectively) suggests a close correspondence in the molecular organization [cf. Sata et al. (1972)] of the VLDL in the two species.

Data on the composition and properties of the VLDL of the squirrel monkey are available from the studies of Illingworth (1975). The fraction from the squirrel monkey contained a lower proportion of triglyceride (30.5%) and a higher proportion of cholesteryl ester (34.0%) than either human or marmoset VLDL. Moreover, the squirrel monkey VLDL also contained a comparatively low proportion of total neutral lipids. However, the apparent differences in the VLDL fractions between the two studies may relate to the different isolation techniques employed. Illingworth (1975) prepared the VLDL by ultracentrifugation in a fixed angle rotor rather than the density gradient procedure used here. In a study of the lipoproteins of the *Cebus* monkey (capuchin, *Cebus albifrons*), Hojnacki et al. (1977) found that the VLDL isolated in the fixed angle rotor was richer in phospholipid and protein and poorer in cholesterol and triglyceride than the VLDL isolated by the density gradient procedure. The chemical composition of the *Cebus* monkey VLDL isolated by the latter technique was characterized by a lower proportion of triglyceride and a higher proportion of protein than the VLDL of either marmoset or man described here. However, comparison of the VLDL fractions of these primates is additionally complicated by differences in diet. The *Cebus* monkeys examined by Hojnacki et al. (1977) were fed a diet supplemented by 15% corn oil and 0.1% cholesterol.

Marmoset and human LDL isolated on the density gradient (M. J. Chapman, S. Goldstein, D. Lagrange, I. Tayeb, and M. H. Laudat, unpublished experiments) and banding with similar hydrated density (within the range 1.027–1.055 g/mL) resembled each other in chemical composition, but, as with VLDL, the marmoset lipoprotein was comparatively deficient in unesterified cholesterol. In addition, marmoset LDL contained a higher proportion of triglyceride (10.9%) than human

LDL (6.5%). However, the total neutral lipid contents were not markedly different (48.3 and 45.3% in marmoset and human LDL, respectively). Taking this together with the similar dimensions of LDL in the two species (representative mean diameters of 210 and 208 Å, respectively), it can be suggested that a similar molecular organization of LDL prevails in marmoset and man.

It is interesting to note that the LDL of the squirrel monkey (Illingworth, 1975) also contained a high proportion of triglyceride (8.5%). In the LDL of this species, however, the total contribution from apolar components was rather low (41.1%) in comparison to marmoset or man. It is possible that this difference in chemical composition reflects a somewhat higher peak hydrated density.

It is pertinent to comment on the heterogeneity of the LDL in New and Old World primates. Thus, despite the paucity of data on this aspect, it is noteworthy that Hill et al. (1975), using analytical ultracentrifugation, could resolve two LDL components in capuchin and some rhesus monkeys. Examination of LDL from the pooled serum of several marmosets (G. L. Mills, personal communication) revealed only a single component with a distribution of flotation rates and a peak flotation rate (S_f 8) indistinguishable from the human lipoprotein.

The impression that the VLDL and LDL of the marmoset conformed closely to their human counterparts was again confirmed when their protein moieties were examined by NaDodSO$_4$–polyacrylamide gel electrophoresis. This technique revealed the single major protein component of high molecular weight (>250 000) typical of human apo-B. Moreover, the use of a monospecific antiserum to human apo-B permitted the detection of an immunologically similar protein in marmoset VLDL and LDL.

Earlier reports from our laboratory (Chapman & Goldstein, 1976; Goldstein et al., 1977) have documented the presence of apo-B-like proteins in the serum LDL of two Old World primates, the baboon (*Papio anubis*) and rhesus (*Macaca mulatta*). Evidence for the existence of an apo-B-like component in the LDL of a New World primate (squirrel monkey) has previously been reported by Illingworth (1975), who noted that a major portion of the LDL apoprotein did not enter the running gel (7.5% polyacrylamide) during electrophoresis at basic pH.

The density distribution of the α-reacting, high-density lipoproteins of marmoset serum differed somewhat from that characteristic of normolipidemic man. Thus, the principal species of marmoset HDL was detected on the gradient with a hydrated density in the range 1.061–1.095 g/mL (Table I); the hydrated density of human HDL$_2$, 1.09 g/mL (Scanu, 1972), falls within these limits. Small amounts of α-reacting, high-density substances were also detected in the (hydrated) density ranges 1.095–1.120 and 1.120–1.150 g/mL; such particles correspond more closely to the human HDL$_3$ class, whose average hydrated density is 1.12 g/mL (Scanu, 1972). Subsequent removal of the marmoset high-density lipoproteins according to their gradient banding pattern led to the recovery of two fractions, HDL-I of d = 1.070–1.127 g/mL, whose hydrated density range thus encompassed that of HDL$_2$ and overlapped that of HDL$_3$ of man, and HDL-II of d = 1.127–1.156 g/mL.

The chemical composition and particle size (mean diameter 100 Å) of HDL-I suggest that it corresponds rather closely to human HDL$_2$ (mean diameter 95–100 Å; Forte & Nichols, 1972). Similar comments apply to HDL-II and human HDL$_3$, whose compositions (Table II; Anderson et al., 1978) and particle sizes [mean diameter of 69 and 70–75 Å (Forte & Nichols, 1972), respectively] are alike. The similarity between the respective human and marmoset HDL subfractions extended to their major apolipoproteins since electrophoresis in NaDodSO$_4$– and urea–polyacrylamide gel systems revealed the presence of marmoset components with electrophoretic mobility and size approaching those of the human A-I and A-II (monomer) proteins (Figures 5 and 6).

Additional evidence suggesting the presence of a protein component analogous to human A-I was obtained in immunodiffusion studies in which a monospecific antihuman A-I antiserum was found to react with marmoset HDL and whole serum. The mobility of the A-II-like component (band 8, parts C and D of Figure 6) of marmoset HDL was not affected by reducing agents, an indication that it lacks the cystine residue which links two identical subunits in the human protein. Such lack of conformity to the human protein has been noted in other nonhuman primates (Edelstein et al., 1973; Blaton et al., 1977).

Only scant information is available on the chemical properties of the HDL of other (normolipidemic) New World primates, and this primarily concerns the chemical composition and apoprotein pattern of squirrel monkey HDL$_2$ and HDL$_3$ (Illingworth, 1975) and the carbohydrate composition and apoprotein pattern of these HDL subclasses in spider monkeys (*Ateles* sp.) (Pargaonkar et al., 1977). Squirrel monkey HDL$_2$ and HDL$_3$ were akin in composition to those of both marmoset (i.e., HDL-I and HDL-II) and man, although their HDL (d = 1.063–1.21 g/mL) apoprotein gel pattern was quite distinct from that of man (Illingworth, 1975). Although spider monkey HDL$_2$ and HDL$_3$ displayed similar apoprotein gel patterns to their human counterparts (Pargaonkar et al., 1977), some marked dissimilarities were detected in the carbohydrate contents of corresponding human and spider monkey apoproteins; the elevated glucosamine and diminished glucose contents of the monkey apo-HDL$_2$ and the high sialic acid and low fucose and glucose contents of its apo-HDL$_3$ are notable (Pargaonkar et al., 1977).

Although the low molecular weight, soluble apolipoproteins of New World primates await isolation and characterization, it may be concluded from their electrophoretic patterns in polyacrylamide disc gel that some substantial differences exist between them and their human counterparts. In the marmoset, for example, analogues to the human C-I and E apolipoproteins may be present, but forms of apo-C-II and apo-C-III (possibly corresponding to bands 11–14, Figure 6) either are of markedly different electrophoretic mobility or are absent.

Thus, comparison of several aspects of the properties of the major serum lipoproteins of marmoset and man has in general revealed a high degree of homology.

A second important consideration is the quantitative aspects of the lipoprotein profile of the marmoset in comparison to man and other primate species. Thus, the concentrations of the major lipoprotein classes (estimated on the basis of the amount of lipoprotein recovered in the respective gradient fractions) in fasted marmosets were as follows: VLDL, 50–90 mg/dL; LDL, 170–280 mg/dL; and HDL, ~350 mg/dL (range 338–408 mg/dL).

The level of LDL is somewhat lower and that of HDL higher in the marmoset than in urban man (Mills & Taylaur, 1971). Nonetheless, the concentrations in *C. jacchus* approach those of *Homo sapiens* more closely than the corresponding values reported for a number of other nonhuman, New World primates (Hill et al., 1975; Hojnacki et al., 1977; Illingworth, 1975; Mills & Taylaur, 1971; Pargaonkar et al., 1977; Srini-

vasan et al., 1972, 1974, 1976). However, at this stage it is important to point out that the diversity of diets used in the studies of different primates makes comparison between the species difficult.

In primates other than the marmoset, lower levels of VLDL have been encountered. Thus, only 3 and 20 mg/dL have been found in the squirrel monkey by Illingworth (1975) and Srinivasan et al. (1974) (as pre-β-lipoprotein), although the latter workers have also reported higher values in the same species (57 mg/dL; Srinivasan et al., 1976). The *Aotus* monkey (*Aotus trivirgatus*) appears exceptional, since elevated VLDL levels (102 mg/dL) were described by Mills & Taylaur (1971). Concentrations consistently less than 50 mg/dL have been found in both spider and *Cebus* monkeys (Srinivasan et al., 1974, 1976).

The average serum LDL level in the marmoset was ~220 mg/dL, a value similar to that (196 mg/dL) estimated by analytical ultracentrifugation (G. L. Mills, personal communication) and consistent with the range of 40–95 mg/dL of cholesterol found in the $d < 1.063$ g/mL fraction in 24 marmosets of both sexes. Rather variable amounts of LDL have been detected in squirrel monkeys, and these range from 104 mg/dL (Hill et al., 1975) to 236 mg/dL (Srinivasan et al., 1976); concentrations toward the bottom of this range have typically been found in species of capuchin monkeys (Hill et al., 1975; Srinivasan et al., 1974). Somewhat higher amounts occur in the spider monkey (240–280 mg/dL; Srinivasan et al., 1974, 1976), which therefore resembles the marmoset to the greatest degree. Among the Old World primates, LDL levels of 97, 129, and 139 mg/dL have been quoted in the rhesus monkey (Chapman & Goldstein, 1976; Mills & Taylaur, 1971; Srinivasan et al., 1976), while slightly lower values (in the range 60–120 mg/dL) have been reported in the baboon (*Papio* sp.) (Blaton & Peeters, 1976; Chapman & Goldstein, 1976; Srinivasan et al., 1976). Similar concentrations are present in the patas monkey (*Erythrocebus patas*) (~100 mg of LDL/dL; Srinivasan et al., 1976); in this group, only the African green monkey (*Cercopithecus aethiops*) appears to have LDL levels, ~200 mg/dL (Srinivasan et al., 1976), comparable to those of the marmoset.

Thus, among the Old and New World primate species, the marmoset, maintained on a low-fat diet, displays a notably high LDL concentration. We consider that this may be a point in favor of the use of this animal in studies of the metabolic factors which control the level of LDL in the circulation.

Other convenient laboratory animals such as the rat, rabbit, and guinea pig, when maintained on low-fat diets without the addition of cholesterol, typically display low levels of LDL (Calvert, 1976; Mills & Taylaur, 1971). Moreover, in such animals the LDL often does not present a clearly defined and relatively homogeneous species but can be found dispersed over a wide range of density and molecular weight (Pescador, 1978).

In the high-density ($d > 1.063$ g/mL) lipoproteins of the marmoset, the amount of cholesterol transported was, on average, only slightly lower than that carried by the low-density ($d < 1.063$ g/mL) substances (60–70 mg/dL as compared to 72–75 mg/dL in the latter). Similar or higher HDL cholesterol levels have been found in squirrel and capuchin monkeys (Hill et al., 1975; Srinivasan et al., 1974), and also in baboon, rhesus, and several other Old World primates (Hill et al., 1975; Kritchevsky, 1970; Srinivasan et al., 1974). Since high-density lipoproteins seldom contain more than ~20% of cholesterol by weight, those findings are entirely consistent with the large amounts of HDL (300–600 mg/dL) generally found in these species (Blaton & Peeters, 1976; Illingworth, 1975; Srinivasan et al., 1974, 1976). On occasion, however, lower HDL concentrations have been detected in some of these species, notably by Hill et al. (1975) in squirrel, *Cebus*, and rhesus monkeys in which 194, 198, and 169 mg/dL HDL was found under control conditions of diet. If the former (higher) values are accepted, then all of the aforementioned nonhuman primate species, i.e., marmoset, squirrel, capuchin, baboon, and rhesus monkeys, differ markedly from man in transporting about half of their total cholesterol in the form of HDL. Thus, in man approximately one-fifth of the total cholesterol is carried in HDL, and HDL concentrations themselves are of the order of 250 mg/dL in normolipidemic males. Only the spider monkey would appear to be exceptional among the New and Old World monkeys cited above, since its HDL and HDL cholesterol levels are about one-half of those of man (Srinivasan et al., 1972, 1974, 1976).

An important difference in the lipoprotein profile between man and the marmoset concerns the relative amounts of HDL subclasses. Thus, in man the HDL_2/HDL_3 ratio is ~1:3 whereas in our study of the marmoset the HDL-I/HDL-II ratio (fractions we have shown to approximate HDL_2 and HDL_3, respectively) is between 3 and 4:1.[4] It is interesting to note that similarly high levels of HDL_2 have been found in the squirrel monkey (Illingworth, 1975).

In conclusion, the major quantitative and qualitative characteristics of the serum lipoproteins of the common marmoset are presently delineated and provide basic knowledge for its future use as an experimental animal in lipoprotein research.

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References

Abell, L. L., Levy, B. B., Brodie, B. B., & Kendall, F. E. (1952) *J. Biol. Chem. 195*, 357–362.

Alaupovic, P. (1972) *Protides Biol. Fluids, Proc. Colloq. 19*, 9–19.

Anderson, D. W., Nichols, A. V., Pan, S. S., & Lindgren, F. T. (1978) *Atherosclerosis 29*, 161–179.

Bartlett, C. R. (1959) *J. Biol. Chem. 234*, 466–468.

Biggs, H. C., Erickson, J. M., & Moorehead, R. (1975) *Clin. Chem. (Winston-Salem, N.C.) 21*, 437–450.

Blaton, V., & Peeters, H. (1976) *Adv. Exp. Med. Biol. 67*, 33–64.

Blaton, V., Vercaemst, R., Rosseneu, M., Mortelmans, J., Jackson, R. L., Gotto, A. M., Jr., & Peeters, H. (1977) *Biochemistry 16*, 2157–2162.

Brown, W. V., Levy, R. I., & Fredrickson, D. S. (1969) *J. Biol. Chem. 244*, 5687–5694.

Calvert, G. D. (1976) *Low-Density Lipoproteins* (Day, C. E., & Levy, R. S., Eds.) pp 281–319, Plenum Press, New York.

Chapman, M. J., & Goldstein, S. (1976) *Atherosclerosis 25*, 267–291.

[4] The level of marmoset "HDL_2" (HDL-I) as obtained from the gradient is an overestimate if the classical definition of HDL_2 [i.e., d = 1.063–1.125 g/mL and hydrated density = 1.12 g/mL (Scanu, 1972)] is adhered to, since its (hydrated) density corresponded to 1.070–1.127 g/mL.

Chapman, M. J., Mills, G. L., & Ledford, J. H. (1975) *Biochem. J. 149*, 423–436.
Chapman, M. J., Goldstein, S., & Laudat, M. H. (1977) *Biochemistry 16*, 3006–3015.
Davis, B. J. (1964) *Ann. N.Y. Acad. Sci. 121*, 404–427.
Edelstein, C., Lim, C. T., & Scanu, A. M. (1973) *J. Biol. Chem. 248*, 7653–7660.
Forte, G. M., & Nichols, A. V. (1972) *Adv. Lipid Res. 10*, 1–41.
Forte, G. M., Nichols, A. V., & Glaeser, R. M. (1968) *Chem. Phys. Lipids 2*, 396–408.
Goldstein, S., Chapman, M. J., & Mills, G. L. (1977) *Atherosclerosis 28*, 93–100.
Gotto, A. M., Jr., Miller, N. E., & Oliver, M. F., Eds. (1978) *High-Density Lipoproteins and Atherosclerosis*, Elsevier, Amsterdam.
Hatch, F. T., & Lees, R. S. (1968) *Adv. Lipid Res. 6*, 2–63.
Havel, R. J., Eder, H. A., & Bragdon, J. H. (1955) *J. Clin. Invest. 43*, 1345–1353.
Hearn, J. P., Lunn, S. F., Burden, F. J., & Pilcher, M. M. (1975) *Lab. Anim. 9*, 125–134.
Herbert, P. N., Schulman, R. S., Levy, R. I., & Fredrickson, D. S. (1973) *J. Biol. Chem. 248*, 4941–4946.
Hiddleston, W. A. (1978) *Recent Advances in Primatology* (Chivers, D. J., & Lane-Petter, W., Eds.) pp 173–182, Academic Press, London.
Hill, P. W., Martin, W. G., & Douglas, J. F. (1975) *Proc. Soc. Exp. Biol. Med. 148*, 41–49.
Hirsch, J., & Ahrens, E. J. (1958) *J. Biol. Chem. 233*, 311–320.
Hojnacki, J. L., Nicolosi, R. J., Llansa, N., & Hayes, K. C. (1977) *Lipids 13*, 88–91.
Illingworth, D. R. (1975) *Biochim. Biophys. Acta 388*, 38–51.
Illingworth, D. R., & Portman, O. W. (1972) *J. Lipid Res. 13*, 220–227.
Illingworth, D. R., Portman, O. W., & Whipple, L. E. (1974) *Biochim. Biophys. Acta 369*, 304–314.
Illingworth, D. R., Whipple, L. E., & Portman, O. W. (1975) *Atherosclerosis 22*, 325–334.
Ingram, J. C. (1975) *Lab. Anim. 9*, 249–256.
Kane, J. P. (1973) *Anal. Biochem. 53*, 350–364.
Kritchevsky, D. (1970) *Trans. N.Y. Acad. Sci. 32*, 821–831.
Lee, D. M. (1976) *Low-Density Lipoproteins* (Day, C. E., & Levy, R. S., Eds.) pp 3–47, Plenum Press, New York.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Mancini, G., Carbonara, A. O., & Heremans, J. F. (1965) *Immunochemistry 2*, 235–254.
Mendoza, S., Lutmer, R. F., Glueck, C. J., Chen, C-Y., Steiner, P. M., Fallat, R. W., & Kashyap, M. L. (1976) *Atherosclerosis 25*, 131–136.
Mills, G. L., & Taylaur, C. E. (1971) *Comp. Biochem. Physiol. B 40*, 489–501.
Ouchterlony, O. (1964) *Immunological Method* (Ackroyd, J. F., Ed.) p 55, Blackwell, London.
Pargaonkar, P. S., Radhakrishnamurthy, B., Srinivasan, S. R., & Berenson, G. S. (1977) *Comp. Biochem. Physiol. B 56*, 293–299.
Pescador, R. (1978) *Life Sci. 23*, 1851–1862.
Portman, O. W., Illingworth, D. R., & Alexander, M. (1975) *Biochim. Biophys. Acta 398*, 55–71.
Portman, O. W., Alexander, M., Tanaka, N., & Soltys, P. (1976) *Biochim. Biophys. Acta 450*, 185–196.
Roeschlau, P., Bernt, E., & Gruber, W. (1974) *Z. Klin. Chem. Klin. Biochem. 12*, 226–231.
Rudel, L. L., Greene, D. G., & Shah, R. (1977) *J. Lipid Res. 18*, 734–744.
Sata, T., Havel, R. J., & Jones, A. L. (1972) *J. Lipid Res. 13*, 757–768.
Scanu, A. M. (1972) *Biochim. Biophys. Acta 265*, 471–508.
Scheidegger, J. J. (1955) *Int. Arch. Allergy Appl. Immunol. 7*, 103–110.
Slack, J. (1969) *Lancet* ii, 1380–1383.
Srinivasan, S. R., Dalferes, E. R., Jr., Ruiz, H., Pargaonkar, P. S., Radhakrishnamurthy, B., & Berenson, G. S. (1972) *Proc. Soc. Exp. Biol. Med. 141*, 154–160.
Srinivasan, S. R., McBride, J. R., Radhakrishnamurthy, B., & Berenson, G. S. (1974) *Comp. Biochem. Physiol. B 47*, 711–716.
Srinivasan, S. R., Smith, C. C., Radhakrishnamurthy, B., Wolf, R. H., & Berenson, G. S. (1976) *Adv. Exp. Med. Biol. 67*, 65–75.
Stone, M., Thorp, J., & Wain, J. (1976) *Atheroscler., Proc. Int. Symp., 4th, 1975*, 702.
Warnick, G. R., & Albers, J. J. (1978) *J. Lipid Res. 19*, 65–76.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.