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Characterization and Comparative Aspects of the Serum Very Low and Low Density Lipoproteins and Their Apoproteins in the Chicken (*Gallus domesticus*)†

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With the Technical Assistance of Dominique Lagrange

ABSTRACT: Sera from young laying chickens, found to be hypertriglyceridemic by serum lipid and lipoprotein analyses, were fractionated by ultracentrifugation into very low (d < 1.006 g/mL) and low density (d 1.006–1.063 and 1.024–1.045 g/mL) lipoproteins (VLDL and LDL). The purity of these lipoprotein fractions was evaluated by electrophoretic, immunological, and electron microscopic techniques; their chemical and physical properties were subsequently determined and compared with those of the corresponding human fractions. While an overall resemblance was evident between each chicken fraction and its human counterpart, minor differences were detected in surface charge, chemical composition, and particle size. Both chicken VLDL and LDL exhibited low surface charge upon electrophoresis; the triglyceride content and particle size of the chicken LDL fractions were greater than those of the corresponding human preparations. Immunological studies revealed a partial identity between the VLDL and between the LDL of chicken and man; quantitative microprecipitation showed the cross-reactivity of chicken and human LDL to amount to about 10%. The chicken lipoproteins possessed a common antigenic determinant and reacted strongly to an antiserum to human apolipoprotein B. The presence of an apolipoprotein B like component in chicken VLDL and LDL was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis of their total apoproteins, which revealed a major component of high molecular weight (>250 000). This component was isolated in the void volume as fraction I upon gel filtration chromatography on Sephadex G-200; the behavior of fraction I on sodium dodecyl sulfate–polyacrylamide gel and its amino acid composition indicate that it is a counterpart of apolipoprotein B in man. Low-molecular-weight components were detected in apo-VLDL and apo-LDL by electrophoretic procedures in polyacrylamide gel; gel filtration chromatography facilitated their isolation as fraction II and showed them to constitute some 50% of apo-VLDL, but rather less of apo-LDL (d 1.006–1.063 g/mL, about 25%; d 1.024–1.045 g/mL, about 20%). The basic character of the fraction II apoproteins was revealed by amino acid analysis and by their low electrophoretic mobility in polyacrylamide gel at pH 8.9. Fraction II contained up to seven components, the most prominent exhibiting molecular weights of 27 000, 18 500–21 400, 13 500–14 000, 8500, and 4000. These data indicate that the major protein component of chicken VLDL and LDL is a counterpart of human apolipoprotein B, although the lower molecular weight components of these avian lipoproteins appear to be more distinct from those of man.

In recent years, avian species have attracted considerable interest as animal models in which to study the mechanisms of estrogen-induced hyperlipidemia (Hillyard et al., 1956; Kudzma et al., 1973; Luskey et al., 1974) and of diet-induced hypercholesterolemia (Hillyard et al., 1956; Kruski and Narayan, 1972). Several aspects of lipid metabolism in birds appear, however, to differ substantially from those typical of man. In particular, avians absorb exogenous fat as VLDL[1] via

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[1] Abbreviations used: VLDL, very low density lipoproteins of d < 1.007 g/mL; LDL, low density lipoproteins, density as defined; HDL, high density lipoproteins, d 1.063–1.21 g/mL; apo-B, apolipoprotein B; EDTA, ethylenediaminetetraacetic acid; SEM, standard error of the mean.

the portal vein (Noyan et al., 1964; Bensadoun and Rothfeld, 1972), rather than in mammals as chylomicrons via the lymphatic system. Furthermore, the major site of lipogenesis in avians is the liver, whereas, in mammals, lipid biosynthesis occurs both in liver and adipose tissue (Favarger, 1965; Leveille et al., 1968).

Despite the documented differences between the metabolism and transport of lipids in mammalian and avian species, there is a paucity of information on the exact nature of the lipid-transporting proteins in the circulatory system of birds. Attention has been focused on the HDL class (Kruski and Scanu, 1975), in which the major apoprotein component is an analogue of the human AI apolipoprotein (Jackson et al., 1976). Whereas various data on the general properties of chicken HDL and apo-HDL are available (Hillyard et al., 1972; Kruski and Scanu, 1975; Jackson et al., 1976), the serum VLDL and LDL of this avian are relatively poorly characterized. Moreover, the nature of their respective apoproteins, i.e., apo-VLDL and apo-LDL, is the subject of some controversy (Hillyard et al., 1972; Hearn and Bensadoun, 1975; Luskey et al., 1974; Chan et al., 1976), while little information is available on the relationship between these apoproteins and those of man. Since our recent studies have shown that, under certain conditions, the low density lipoproteins of chicken and human serum may cross-react immunologically (Goldstein and Chapman, 1976), whereas Hillyard et al. (1972) have suggested that the serum apolipoproteins in mammals and avians are chemically and immunologically nonidentical, it was of some interest to determine the biochemical origin of the immunological cross-reactivity in chicken and human LDL.

The present report describes the characterization and some comparative aspects of the very low density and low density lipoproteins from hypertriglyceridemic chicken serum. In addition, the principal physical and chemical properties of the major apolipoprotein of these lipoprotein classes in *Gallus* are compared with those of its human counterpart, i.e., apolipoprotein B.

Materials and Methods

Blood Samples. Pooled blood samples (100–200 mL), drawn from 2–4 fed laying Rhode Island Red hens (8 to 11 months of age), were supplied on the same day by Laboratoire Sorga, Plaine St. Denis, France. Human serum was separated from venous blood drawn from healthy normolipidemic adults, without restriction of blood groups. Serum was separated following coagulation by low-speed centrifugation at 4 °C; isolation of lipoproteins was commenced immediately.

Preparation of Serum Lipoproteins. Serum was fractionated by ultracentrifugation according to established procedures (Havel et al., 1955). The VLDL were isolated as the substances of $d < 1.006$ g/mL; LDL was isolated in the conventional density interval of 1.006–1.063 g/mL and also in the narrower density range of 1.024–1.045 g/mL. As a precaution against the proteolytic degradation of these lipoproteins which might result from microbial growth during their isolation and storage, all salt solutions contained 0.02% sodium azide and 0.005% merthiolate, in addition to 0.04% EDTA (Chapman and Kane, 1975). The nonprotein solvent densities of such solutions were determined pycnometrically at 20 °C.

Ultracentrifugation was normally performed at 6 °C in Beckman L3-50 and L3-40 preparative ultracentrifuges.

Chicken and human VLDL were separated as follows: 7–10 mL of serum was overlaid with NaCl solution of density 1.007 g/mL to a final volume of 12.5 mL and centrifuged in a Spinco type 40 rotor at 40 000 rpm (106 000*g*) for 20 h. The floating VLDL were removed from each tube in a volume of 1–2 mL by aspiration; the pooled VLDL was subsequently washed by a single recentrifugation at d 1.006 g/mL under the same conditions (Herbert et al., 1975).

Following aspiration of floating VLDL subsequent to the initial centrifugation at d 1.006 g/mL, the next 2 mL in each tube was discarded; the density of the bottom fraction (infranatant I) was then raised either to 1.024 g/mL (for isolation of the d 1.024–1.045 g/mL LDL fraction) or to 1.063 g/mL (for isolation of LDL of d 1.006–1.063 g/mL). Density adjustments were made in all cases by addition of the appropriate volume of a d 1.338 g/mL NaCl-KBr solution.

Isolation of LDL of d 1.024–1.045 g/mL from infranatant I (after its adjustment to d 1.024 g/mL) was carried out as previously described (Chapman and Goldstein, 1976); the LDL fraction of d 1.006–1.063 g/mL was separated from infranatant I by a similar procedure. It is noteworthy that, while the human LDL's were seen as yellow-orange floating layers, those from chicken appeared opalescent.

LDL fractions were dialyzed exhaustively at 4 °C against a solution containing 0.05 M NaCl, 0.04% EDTA, 0.02% sodium azide, and 0.005% merthiolate at pH 8.6.

Purity of Lipoprotein Fractions. The purity of the VLDL and LDL preparations from the two species was evaluated as follows.

(*1*) *By Agarose Gel Electrophoresis.* VLDL and LDL fractions each migrated as a single band in the absence of any additional bands. In all cases, the mobility of VLDL was pre-β and that of LDL was β.

(*2*) *By Immunodiffusion and Immunoelectrophoresis Employing Rabbit Antisera to the Homologous Whole Serum, Homologous Albumin, Homologous LDL, and to Human VLDL.* Each LDL preparation gave a single precipitation line which stained (separately) for protein, lipid, and carbohydrate and which was characteristic of β lipoprotein. Upon reaction against antiserum to either the homologous whole serum or to human VLDL, both chicken and human VLDL gave a single precipitation line; this line was situated in the β region on immunoelectrophoresis but was of slower mobility than that of the LDL fractions. None of the chicken or human fractions reacted with antiserum to homologous albumin.

(*3*) *By negative stain electron microscopy,* the chicken and human LDL preparations contained no more than 2% of particles with diameters less than 150 Å, suggesting no significant contamination by HDL.

These observations establish the purity of the serum VLDL and LDL preparations from chicken and man and indicate that no other serum proteins were present as contaminants.

Characterization of Lipoproteins. Chemical Analysis. The lipid and protein components of a sample (2–10 mg) of each lipoprotein preparation were separated by solvent extraction at 4 °C; the method employed was a modification (Chapman et al., 1975) of that of Brown et al. (1969) and resulted in the loss of <1% of the total protein in the solvent phase, while the protein precipitate contained not more than 1% by weight of phospholipid. The protein precipitate was finally washed with diethyl ether, dried under nitrogen, dissolved in 1 N NaOH and estimated by the procedure of Lowry et al. (1951), employing bovine serum albumin (Sigma) as the calibration standard.

The various lipid components in the combined solvent extracts were separated and quantified according to the series of procedures outlined by Mills et al. (1972).

Agarose Gel Electrophoresis. Electrophoresis in agarose gel was carried out according to the principles of Noble's method

TABLE I: Concentrations of Serum VLDL, LDL, and HDL in Chicken Serum.

Lipoprotein class	Lipoprotein concn[a] (mg/100 mL serum)				
	VLDL		LDL		HDL
S_f 1.063 range	400–100	100–20	20–12	12–0	
Density interval (g/mL)	<1.006		1.006–1.019	1.019–1.063	1.063–1.21
Sample 1	88.4	335.7	91.5	32.9	78.1
Sample 2	142.2	651.6	55.6	19.2	150.8

[a] Concentrations were determined by analytical ultracentrifugation at a solvent density of 1.063 g/mL for VLDL and LDL, and at 1.21 g/mL for HDL. The procedures employed were those of Mills et al. (1972).

(Noble, 1968), using kits prepared by Bio-Rad. The gels were stained with Sudan black.

Electron Microscopy. Lipoprotein preparations containing up to 0.2 mg protein/mL were negatively stained with 2% potassium phosphotungstate at pH 7.4 as described by Forte et al. (1968).

Stained preparations were examined at 60 kV with a Philips EM 300 electron microscope fitted with a decontamination device. For the determination of the distribution of particle diameters, 100–500 particles were measured with a microcomparator on each of a series of 2–5 micrographs. Only freely dispersed particles, which were not obviously fractured, were measured.

Immunological Methods. Antisera to human LDL (*d* 1.024–1.045 g/mL) and to human apolipoprotein B were prepared in rabbits of the Fauve de Bourgogne strain as previously described (Chapman and Goldstein, 1976). The production of antisera to human VLDL and to chicken LDL (*d* 1.006–1.063 g/mL) was performed in the same manner, using as immunogens 0.6 and 1.6 mg of protein of each fraction, respectively. Rabbit antisera to whole chicken serum and to hen ovalbumin were supplied by Behringwerke AG, Marburg Lahn, West Germany.

Double immunodiffusion and immunoelectrophoresis were carried out by the techniques of Ouchterlony (1964) and Scheidegger (1955), respectively. For the quantitative precipitation reaction, we employed the microimmunoprecipitation procedure of Maurer (1971) under conditions previously described (Goldstein and Chapman, 1976).

In the quantitative precipitation reactions, antisera to human and chicken LDL were reacted with human and chicken LDL and VLDL. The degree of cross-reactivity of each species of LDL was determined as the amount of protein in the immunoprecipitate formed between it and the heterologous antiserum expressed relative to that obtained in the reaction of the same LDL with its homologous antiserum. The protein content of immunoprecipitates was determined by the method of Lowry et al. (1951).

Isolation and Characterization of Apolipoproteins. The delipidated apolipoprotein residues of VLDL and LDL, prepared as outlined under Chemical Analysis, were dissolved in a sodium phosphate buffer (0.1 M) containing 1% (w/v) sodium dodecyl sulfate and 1% β-mercaptoethanol at pH 8.0 (solution S). Since this buffer is the same as that employed in the procedure of Weber and Osborn (1969) for sodium dodecyl sulfate–polyacrylamide gel electrophoresis, samples could be taken directly for such studies: electrophoresis of 100–200 μg of protein was performed in 3.3, 7.5, and 10% gels. Samples were pretreated at 90 °C for 3 min rather than for 2 h at 37 °C as described by Weber and Osborn (1969). Protein fixation and staining were carried out as described by Chapman et al. (1975). Additional samples of the detergent-solubilized apoproteins were taken for immunological studies.

The whole apoprotein residues were also examined in the polyacrylamide gel electrophoretic system described by Takayama et al. (1964). The residues were completely soluble in the phenol–acetic acid–water solution employed; final gel concentrations used were 3.3 and 7.5%.

Electrophoresis of apoprotein components soluble in tetramethylurea was performed in 7.5% polyacrylamide gels (pH 8.9) containing 8 M urea by the modification of Kane (1973) of the procedure of Davis (1965). Gels were stained with Coomassie brilliant blue and scanned densitometrically.

The detergent-solubilized apoproteins (up to 5 mg) were fractionated by gel filtration chromatography on Sephadex G-200 by a procedure based on that of Herbert et al. (1973) for separation of the apoproteins of human serum VLDL. Our conditions of fractionation have been described elsewhere (Chapman and Goldstein, 1976). Column fractions were lyophilized and dissolved in a volume of double-distilled water to give a final concentration of sodium decyl sulfate between 5 and 15 mM (0.4% w/v). Complete and rapid solubilization of all fractions (either chicken or human) was obtained with this procedure. Samples of each fraction were then taken for immunological studies, for amino acid analysis and for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (after adjustment of the sodium dodecyl sulfate buffer concentration).

Amino Acid Analyses. Samples (250–500 μg) of the dried apoprotein preparations were hydrolyzed as previously reported (Chapman et al., 1975). Amino acid analysis of the hydrolysates was performed on a JEOL JLC 5AH amino acid analyzer employing a two-column program (Moore et al., 1958).

Results

Serum Lipid and Lipoprotein Concentrations. In four samples of pooled chicken sera examined, the mean total serum cholesterol and triglyceride concentrations ± SEM were 101 ± 28 and 457 ± 144 mg/100 mL serum, respectively. The range in serum triglycerides (240–672 mg/100 mL) was substantially greater than that in cholesterol (58–125 mg/100 mL), suggesting some variation in the degree of hypertriglyceridemia in the donor animals.

Determination of the quantitative distribution of the serum lipoproteins by analytical ultracentrifugation at solvent densities of either 1.063 or 1.21 g/mL indicated that the predominant lipoprotein class in these nonfasting chickens was that of VLDL (Table I). It is noteworthy that only a small proportion of the VLDL in these avians was present as particles

TABLE II: Mean Weight % Chemical Composition of Chicken and Human LDL.[a]

Species: Density (g/mL):	Chicken 1.024–1.045	Man[b] 1.024–1.045	Chicken 1.006–1.063	Chicken[c] 1.006–1.063	Man[c] 1.006–1.063
Component	(3)		(3)		
Cholesteryl ester	34.9 ± 0.3	38.9 ± 0.4	28.6 ± 6.1	27.8	38.0
Free cholesterol	13.4 ± 0.6	8.8 ± 0.2	8.3 ± 1.2	6.4	9.0
Triglyceride	11.3 ± 2.0	6.3 ± 2.2	19.4 ± 4.3	22.1	11.2
Phospholipid	18.8 ± 0.5	22.8 ± 1.9	21.2 ± 2.9	20.2	22.1
Protein	21.6 ± 1.3	23.2 ± 1.7	22.5 ± 4.8	23.5	20.9

[a] Values are the means ± SD of the number of preparations given in parentheses. [b] Data in man from Chapman and Goldstein (1976). [c] Data in chicken and man from Mills and Taylaur (1971).

of $S_f > 100$, some 80% being detected as molecules of S_f 20–100. Furthermore, the major proportion (>70%) of the LDL tended to be distributed rather broadly within the S_f range 12–20 (i.e., in the density interval 1.006–1.019 g/mL) (profiles not shown). The concentration of low density substances in the S_f range 0–12, in which the majority of LDL in normal man is present (Mills and Taylaur, 1971), was therefore low (<50 mg/100 mL serum) (Table I). The peak S_f of the profile of very low density and low density substances was in the range 14–30.

Characterization and Comparative Aspects of the Native VLDL and LDL. Agarose Gel Electrophoresis. Electrophoresis in agarose gel showed that both chicken VLDL and LDL had slower mobilities than their human counterparts.[2] Thus chicken VLDL migrated as a single band; the ratio of the mobilities (taking the migration distance of human VLDL as 1.0) was 0.81:1.0. The chicken LDL fractions (density 1.006–1.063 g/mL and 1.024–1.045 g/mL) each migrated as single narrow bands of essentially identical mobility; their mobility was rather less than that of the corresponding fractions of human LDL. The relative mobility of the chicken LDL fractions to that of man was: for LDL of d 1.024–1.045 g/mL, 0.46:1.0, and for LDL of d 1.006–1.063 g/mL, 0.41:1.0 (two preparations each).

Chemical Composition. The mean weight percent chemical compositions of the chicken LDL of densities 1.024–1.045 and 1.006–1.063 g/mL are compared with the corresponding human fractions in Table II; chicken VLDL were not analyzed. The composition of the chicken fraction of d 1.024–1.045 g/mL resembled that of its human counterpart, although its triglyceride content was some twofold greater; the total contents of neutral lipid in the fractions from the two species were, however, very similar. A higher proportion of triglyceride was found in the chicken LDL of density 1.006–1.063 g/mL; the composition of this fraction was similar to that reported by Mills and Taylaur (1971). As indicated by the high standard deviations, the compositions of our (three) preparations were rather variable, and probably result from differences in the quantitative distribution of the LDL from the various birds used as blood donors. While an overall resemblance between the chicken and human LDL (d 1.006–1.063 g/mL) was apparent, the chicken fraction was rather richer in triglyceride and poorer in cholesteryl ester than its human counterpart; the total neutral lipid content (cholesteryl ester and triglyceride) in these fractions was essentially identical.

Electron Microscopic Studies. Electron microscopic examination of negatively stained chicken VLDL showed them to be relatively homogeneous in size, to be approximately spherical when free standing, and to deform upon contact (Figure 1a). Their mean diameter was 340 Å and the range in particle size was 270–540 Å; these dimensions were determined on one preparation considered representative of three.

The diameters of particles in LDL of d 1.024–1.045 g/mL were rather uniformly distributed over a range from about 180

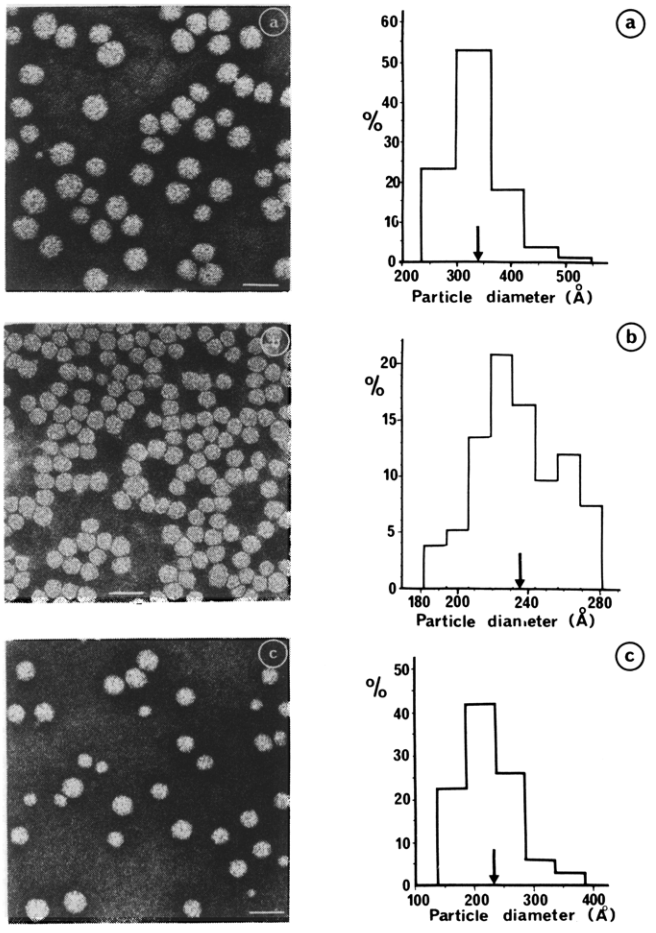

FIGURE 1: Particle characteristics and diameters in representative samples of chicken VLDL and LDL. At left is shown a representative electron micrograph of each fraction stained negatively with 2% phosphotungstate; each bar represents 500 Å; at right is shown the frequency distribution of lipoprotein particles determined from electron micrographs; the arrows indicate the mean diameters. The distributions exclude up to 5% of the occasional particles detected at either extreme of the ranges. Fractions are (a) Chicken VLDL, d < 1.006 g/mL, (b) chicken LDL, d = 1.024–1.045 g/mL, and (c) chicken LDL, d = 1.006–1.063 g/mL.

[2] Reference to the human counterpart(s) implies that the corresponding VLDL or LDL fraction was isolated from normolipidemic human serum unless otherwise stated.

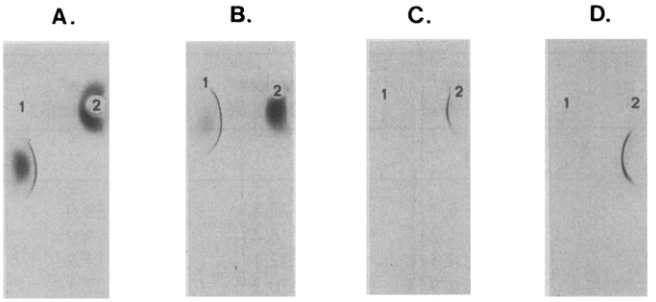

FIGURE 2: Immunoelectrophoretic pattern of human and chicken LDL. Slides A and B: antiserum to human LDL (140 μL, central trough). Slides C and D: antiserum to chicken LDL (140 μL, central trough). Slides A and C: well 1, human LDL (d = 1.024–1.045 g/mL); well 2, chicken LDL (d = 1.024–1.045 g/mL). Slides B and D: well 1, human LDL (d = 1.019–1.063 g/mL); well 2, chicken LDL (d = 1.006–1.063 g/mL). About 60 μg of chicken and human LDL were used in each case. Slides were stained for lipid with Sudan black.

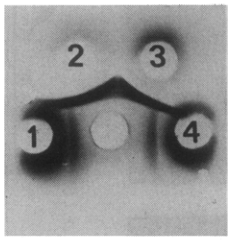

FIGURE 3: Relationship of human and chicken VLDL as revealed by immunodiffusion. Antiserum to chicken LDL (25 μL) in central well. Peripheral wells: 1 and 4, human VLDL (16 μg of protein); 2 and 3, chicken VLDL (60 μg of protein). The slide was stained for lipid.

to 280 Å; some 75% were between 200 and 260 Å (Figure 1b). Their mean diameter was 234 Å, in contrast to the corresponding particles in man which are smaller (mean diameter 217 Å; Chapman and Goldstein, 1976). The electron micrographs gave no indication of a subunit structure (Figure 1b). As in VLDL, freely dispersed molecules were approximately spherical in shape; LDL were, however, less subject to deformation upon contact than the triglyceride-rich lipoproteins.

The range of particle diameters in LDL of d 1.006–1.063 g/mL (160–380 Å) was greater than that in the corresponding fraction isolated in a narrower density interval (i.e., 1.024–1.045 g/mL) (Figure 1c); the mean diameters in the two preparations were, however, very similar (234 and 237 Å, Figures 1c and 1b, respectively). It is of note that a few particles (about 3%) in this fraction were of VLDL size (i.e., >350 Å), thereby indicating an overlap in their particle contents.

Immunological Studies. Immunoelectrophoresis and Immunodiffusion. Upon comparison of the LDL by immunoelectrophoresis against both homologous and heterologous LDL antisera, both chicken LDL fractions presented a slower migration than that of the corresponding human LDL (Figure 2). Against its homologous antiserum, chicken LDL of density 1.006–1.063 g/mL presented three precipitation lines which were located in the β region and all stained for lipid.

Immunodiffusion of human and chicken LDL against homologous and heterologous antisera revealed a partial identity between them (not shown).

In all cases, chicken LDL separated in the density interval 1.006–1.063 g/mL reacted more strongly (for equivalent amounts of protein) than the fraction of density 1.024–1.045 g/mL.

Upon immunodiffusion, human and chicken VLDL reacted

FIGURE 4: Immunodiffusion pattern of human and chicken VLDL and LDL upon reaction with an antiserum to human apolipoprotein B. Slides A and B: central well, antiserum (25 μL) to human apo-B. Slide A: peripheral wells 1 and 4, human VLDL (16 μg of protein); 2 and 3, chicken VLDL (60 μg of protein). Slide B: peripheral wells 1 and 4, human LDL (d = 1.024–1.045 g/mL; 60 μg of protein); 2, chicken LDL (d = 1.024–1.045 g/mL); 60 μg of protein); 3, chicken LDL (d = 1.006–1.063 g/mL; 60 μg of protein); 5, chicken whole serum, 140 μg of protein. The slide was stained for lipid with Sudan black.

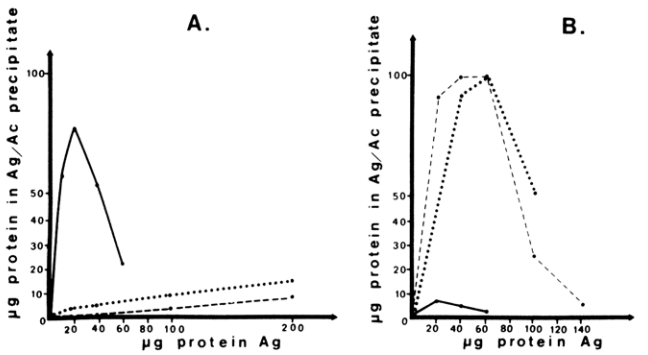

FIGURE 5: Quantitative course of the precipitin reaction between human LDL, d = 1.024–1.045 g/mL (—); chicken LDL, d = 1.006–1.063 g/mL (- -); and chicken LDL, d = 1.024–1.045 g/mL (···) with antiserum to human LDL (course A) and antiserum to chicken LDL (course B). Course A: One-half milliliter of fivefold-diluted antiserum to human LDL was reacted with increasing amounts of each LDL. Course B: One-half milliliter of threefold-diluted antiserum to chicken LDL was reacted with increasing amounts of each LDL.

very strongly against antiserum to chicken LDL, and exhibited a partial identity (Figure 3). In contrast, reaction of chicken VLDL against heterologous antisera (to human VLDL and to human LDL) resulted in the formation of weak precipitin lines (not shown).

Immunodiffusion of chicken and human VLDL and LDL against antiserum to human apolipoprotein B revealed strong precipitation reactions in each case (Figure 4); these reactions showed a partial identity between the VLDL and between the LDL of the two species (chicken LDL reacted rather weakly). It is of note that whole chicken serum reacted strongly against this antiserum. The diffuse staining around the wells is due to the large amounts of VLDL and LDL employed to produce the reactions. These results were confirmed by immunoelectrophoresis.

Quantitative Microimmunoprecipitation Reactions. The above-mentioned reactivities and cross-reactivities of human and chicken LDL with their homologous and heterologous antisera were quantified by precipitation tests in an aqueous system. The characteristic courses for the reaction of the LDL from each species against these antisera are shown in Figure 5. Such reactions revealed a mean cross-reactivity of about 10% between the respective LDL's. It is noteworthy that the chicken LDL fraction of density 1.024–1.045 g/mL reacted to a greater degree against its homologous antiserum than did the fraction of density 1.006–1.063 g/mL.

Quantitative precipitin tests were also performed with

TABLE III: Amino Acid Composition of the Serum VLDL and LDL in Chicken (Moles of Each Amino Acid/100 Moles of Amino Acid Residues).[a]

Species:	Chicken	Chicken	Chicken
Fraction:	Apo-VLDL	Apo-LDL	Apo-LDL
Density (g/mL):	<1.006	1.024–1.045	1.006–1.063
Amino acid			
Lys	8.1	7.6	8.4
His	1.5	1.7	1.5
Arg	5.7	4.6	4.6
Asp	11.7	12.5	13.7
Thr	5.3	6.7	6.6
Ser	6.3	9.0	8.8
Glu	12.0	11.9	12.0
Pro	3.4	4.3	3.1
Gly	5.2	6.0	4.7
Ala	8.2	7.5	7.1
Val	6.2	4.8	5.0
Met	1.4	1.4	1.6
Ile	5.8	4.4	5.4
Leu	11.5	10.4	10.6
Tyr	3.6	3.2	3.0
Phe	4.1	4.0	3.8

[a] Values are the means of duplicate analyses of each of two different preparations.

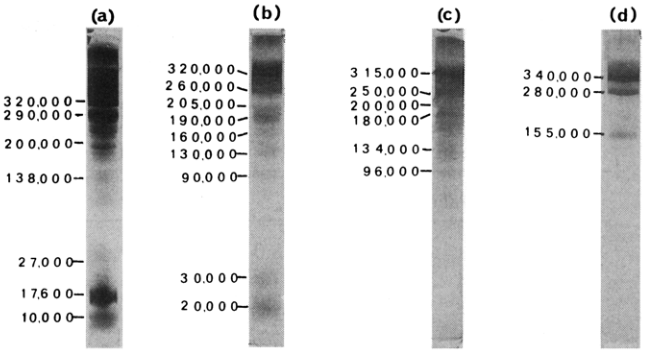

FIGURE 6: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of chicken and human apoproteins on 3.3% acrylamide gels. The molecular weights of the respective bands were determined on a calibration curve resulting from simultaneous electrophoresis of a series of purified, standard proteins. Samples are (a) chicken apo-VLDL, $d < 1.006$ g/mL; (b) chicken apo-LDL, $d = 1.006$–1.063 g/mL; (c) chicken apo-LDL, $d = 1.024$–1.045 g/mL; and (d) human apo-LDL, $d = 1.024$–1.045 g/mL.

human and chicken VLDL, but no conclusive results were obtained, regardless of the antiserum employed. This was probably a result of the high lipid content and low density of the VLDL, which may interfere with the centrifugal isolation of its immunoprecipitate.

Characterization of Apo-VLDL and Apo-LDL. The total protein moieties of chicken serum VLDL and LDL were incompletely soluble in aqueous buffers such as 0.01 M Tris containing 8 M urea at pH 8.0; the residues were, however, totally soluble in buffers containing a detergent such as sodium dodecyl sulfate (e.g., 20 mM).

The amino acid compositions of the total apoprotein from chicken and human VLDL and LDL preparations are presented in Table III; all fractions were rich in aspartic and glutamic acids and leucine. There was an overall resemblance between our data and those reported by Hillyard et al. (1972) for chicken apo-VLDL. The amino acid compositions of chicken and human apo-VLDL are similar. The apo-LDL fraction (d 1.024–1.045 g/mL) from chicken also resembles that of man (fraction LP-B-IV; Lee and Alaupovic, 1970) in amino acid profile. The compositions of chicken apo-LDL of d 1.024–1.045 g/mL and of 1.006–1.063 g/mL were essentially indistinguishable; they were, however, substantially richer in aspartic acid (2% or more) than that of d 1.006–1.063 g/mL reported by Hillyard et al. (1972).

Immunoelectrophoretic comparison of totally delipidated chicken VLDL and LDL with their human counterparts, employing both homologous and heterologous antisera, revealed that the apoproteins of each species reacted only with homologous antisera.

Examination of the apoproteins of VLDL and LDL by electrophoresis in sodium dodecyl sulfate–polyacrylamide gel showed their major component to be of high molecular weight (>250 000) (Figure 6). The patterns displayed by chicken apo-VLDL and apo-LDL were particularly alike in the zones of the gels in which components of molecular weight greater than about 100 000 migrated. In contrast, differences were evident in the lower molecular weight bands of these apoproteins. Thus, whereas apo-VLDL displayed prominent low-molecular-weight components of 27 000, 17 600, and 10 000, only bands corresponding to the first two could be detected in apo-LDL of d 1.006–1.063 g/mL; such bands were undetectable in apo-LDL of d 1.024–1.045 g/mL. The major polypeptide(s) of human apo-LDL (d 1.024–1.045 g/mL) was of high molecular weight; smaller components were not seen. It is noteworthy that a 3.3% monomer concentration was used in these experiments to facilitate entry into the gels of high-molecular-weight material in particular; such a concentration does not resolve components of molecular weight <30 000 satisfactorily and they are typically visualized as rather diffuse bands. Any molecular weight estimates made in this range may therefore be taken at best as approximate.

Electrophoresis of chicken and human apo-VLDL and apo-LDL (solubilized in phenol–urea–acetic acid), in 3.3% polyacrylamide gels according to Takayama et al. (1964), resulted in patterns which were essentially identical with those described above in the sodium dodecyl sulfate system. Use of 7.5% (or higher) gels resulted in minimal migration of the components of high molecular weight, large amounts of material remaining close to the top of the gel.

The tetramethylurea-soluble components of chicken apo-VLDL and apo-LDL were examined by electrophoresis in 7.5% polyacrylamide gel containing 8 M urea (Kane, 1973). All of the detectable components were of relatively low mobility and thus basic in character (Figure 7). The predominant bands (c and f) in apo-VLDL each accounted for 30–50% of the total on a densitometric basis; component f stained rather diffusely, while bands a, e, and h were present in trace amounts (5% or less). Five to eight bands were usually resolved in apo-VLDL; in apo-LDL of d 1.006–1.063 g/mL only four of these (b, c, d, and f) were present, while in apo-LDL of d 1.024–1.045 g/mL, band c was the sole detectable component (Figure 7). Comparison of the chicken pattern with that given by human VLDL revealed that the major soluble chicken component (c) migrated similarly to the arginine-rich peptide. No chicken components were detected with mobilities comparable to those of the human C-II and C-III apolipoproteins.

Characterization of Fractionated Apolipoproteins. Gel filtration chromatography on Sephadex G-200 with sodium decyl sulfate of either apo-VLDL or apo-LDL (previously solubilized in sodium dodecyl sulfate) revealed two major fractions in each case (Figure 8); the only difference between

TABLE IV: Comparison of the Amino Acid Composition of Fraction I and Fraction II from Chicken Serum VLDL and LDL (Moles of Each Amino Acid/100 Moles of Amino Acid Residues).[a]

Lipoprotein class:	VLDL	LDL	LDL	VLDL	LDL	VLDL[b]	VLDL[b]
Density (g/mL):	<1.006	1.006–1.063	1.024–1.045	<1.006	1.006–1.063	<1.006	<1.006
Fraction:	I	I	I	II	II	A	B
Amino acid							
Lys	8.0	9.3	9.4	7.0	11.8	8.1	5.9
His	1.5	1.7	1.6	0.3	0.4	1.5	0
Arg	3.9	4.2	4.4	7.3	7.5	4.0	7.8
Asp	12.2	11.8	12.0	11.7	10.5	11.2	10.6
Thr	6.6	6.6	6.8	6.5	5.5	6.7	6.4
Ser	9.0	8.5	8.9	6.0	6.6	8.4	5.6
Glu	11.5	11.4	11.9	11.8	12.2	12.1	12.1
Pro	4.1	4.1	3.7	3.4	2.7	3.9	1.9
Gly	5.0	5.1	4.8	5.7	4.8	4.6	4.7
Ala	7.3	7.1	6.8	10.5	9.4	6.4	10.1
Val	4.7	5.1	4.9	6.6	7.0	4.7	8.0
Met	2.3	2.0	1.4	1.1	0.8	3.0	1.0
Ile	4.6	5.2	5.1	4.1	4.8	5.4	5.0
Leu	10.6	10.1	10.5	10.7	10.1	10.9	12.1
Tyr	3.7	3.3	3.2	3.8	2.9	3.8	4.4
Phe	5.1	4.5	4.5	3.5	2.8	5.3	2.4
Cys	nd	nd	nd	nd	nd	nd	1.1
Trp	nd	nd	nd	nd	nd	nd	0.8

[a] Values are the means from duplicate analyses of two different preparations of fractions I and of fraction II in each case. [b] Data from Chan et al. (1976). nd, not determined.

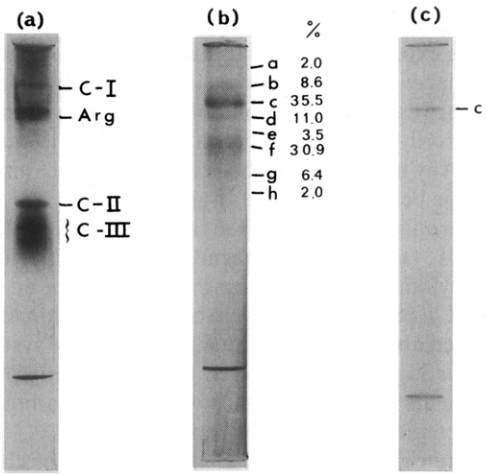

FIGURE 7: Polyacrylamide gel electrophoretic patterns of tetramethylurea-soluble apolipoproteins of chicken and human serum VLDL and of chicken LDL. Samples are (a) human VLDL, $d < 1.006$ g/mL; (b) chicken VLDL, $d < 1.006$ g/mL; and (c) chicken LDL, $d = 1.024$–1.045 g/mL. The 7.5% acrylamide gels contained 8 M urea at pH 8.9. Bands in chicken samples are labeled a–h; those in human VLDL are labeled according to the nomenclature of Alaupovic (1972). Figures in parentheses indicate % densitometric area for each band.

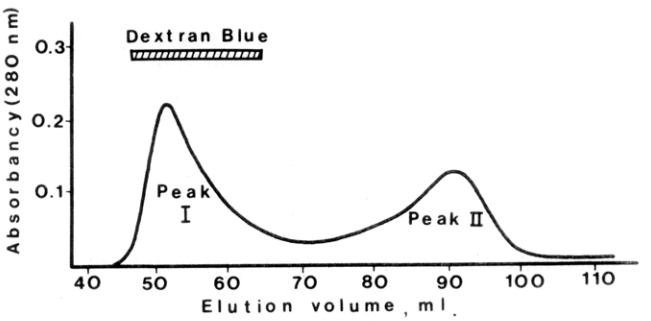

FIGURE 8: Elution profile obtained upon gel filtration chromatography of chicken apo-VLDL on Sephadex G-200 in 5 mM sodium decyl sulfate and 0.01 M Tris at pH 8.0. Two milligrams of protein, dissolved in solution S (containing 1% sodium dodecyl sulfate), was applied to the column (60 × 0.9 cm).

the elution profiles was in the relative proportions of their two component peaks.

Fraction I of apo-VLDL and apo-LDL was eluted in the column void volume and was coincident with blue dextran 2000. In both VLDL and LDL, fraction I was the major component; on the basis of protein recovery from the columns, it constituted 45.6–58.5% (mean 50.8 ± 8.8) of apo-VLDL, whereas in apo-LDL of d 1.006–1.063 and of d 1.024–1.045 g/mL, it amounted to 66.4–83.0% and to 73.1–89.7% of the total, respectively. Upon electrophoresis in sodium dodecyl sulfate–polyacrylamide gel (3.3%), fraction I was typically resolved into a varying number of bands ranging in molecular weight from about 160 000 to 450 000 (Figure 9). A very similar pattern was seen in human apolipoprotein B which had been isolated under the same conditions. The amino acid composition of fraction I from chicken VLDL and LDL is presented in Table IV. The compositions of fraction I from chicken VLDL and LDL (d 1.006–1.063 and d 1.024–1.045 g/mL) resembled each other closely, the only amino acid varying by greater than 1% between them being lysine (range 8.0–9.4%). Thus, fraction I was typically rich in lysine, aspartic and glutamic acids, and leucine. Chicken fraction I and human apo-B (Chapman and Goldstein, 1976) are basically alike in amino acid profile.

The second peak eluted from the Sephadex G-200 column following chromatography of apo-VLDL (Figure 8) contained several apoproteins, which were resolved by electrophoresis in sodium dodecyl sulfate–polyacrylamide gel (Figure 9c). Employing a 7.5% gel concentration, three bands were consistently resolved in fraction II; the first two exhibited molecular weights of 21 300 and 13 700, while the third was diffuse and estimated as 10 000 or less. Use of a higher acrylamide concentration (10%) permitted further resolution of this region into components with molecular weights of 4000 and 8500.

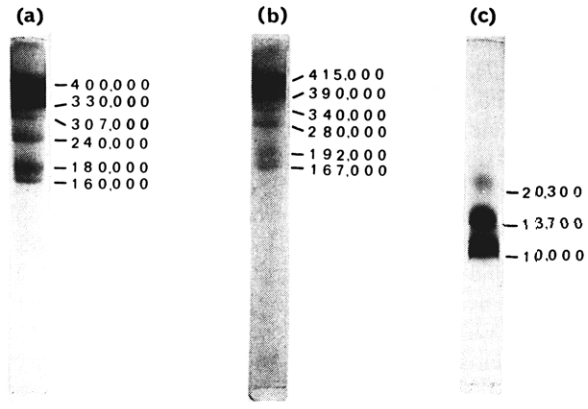

FIGURE 9: Electrophoretic patterns in sodium dodecyl sulfate-polyacrylamide gel of column fractions I and II following gel chromatography on Sephadex G-200. Patterns: (a) Apolipoprotein B from human apo-LDL (d = 1.024–1.045 g/mL) (3.3% gel); (b) fraction I from chicken apo-LDL (d = 1.024–1.045 g/mL) (3.3% gel) and (c) fraction II from chicken apo-VLDL (7.5% gel). Molecular weights were determined from a series of purified proteins which were electrophoresed simultaneously.

Bands of molecular weight similar to those resolved in fraction II from apo-VLDL (in 7.5% acrylamide) were also detected in the corresponding fraction from apo-LDL of density 1.006–1.063 g/mL. These low-molecular-weight components were not further purified. The amino acid compositions of fraction II from chicken VLDL and LDL were markedly alike (Table IV), although the lysine content of the LDL-derived preparation was almost 5% higher than that from VLDL. Both were rich in arginine, aspartic and glutamic acids, alanine, and leucine, and almost devoid of histidine. Fraction II appeared to share certain aspects of its amino acid composition with the human C-I apolipoprotein and others with the arginine-rich peptide (Shore and Shore, 1972).

The isolation and characterization of two major fractions from the serum apo-VLDL of mature laying hens was described by Chan et al. (1976) during the preparation of this report. The first fraction (A) was eluted at the void volume from a Sephadex G-150 column and also resembled apo-B of human VLDL in its behaviour on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Upon comparison of the amino acid composition of fraction A with those of our fractions I (from both VLDL and LDL) (Table IV) and of human apolipoprotein B (Chapman and Goldstein, 1976), a marked resemblance was evident. Their second fraction (B) contained one major polypeptide; traces of a second component were detected. The amino acid composition of fraction B was compared with that of the corresponding fractions (II) isolated in the present study (Table IV); their profiles are very similar.

Discussion

In the present study, we paid particular attention to the purity of our preparations since both Hillyard et al. (1972) and Hearn and Bensadoun (1975) have noted an incomplete separation of chicken lipoproteins (primarily cross-contamination of LDL and HDL). No contamination of chicken LDL, either by HDL or by chicken serum proteins, could be detected by agarose gel electrophoresis, immunological, or electron microscopic investigations.

The concentrations of VLDL and LDL in our hypertriglyceridemic young laying hens exhibited considerable variation (Table I). In general, however, they were similar to those reported by Hillyard et al. (1972) in mature birds. In both cases, VLDL of S_f 20–100 were the predominant lipoprotein species. By contrast, the chickens studies by Mills and Taylaur (1971) exhibited higher levels of S_f 100–400 substances. These variations probably result from differences in the hormonal/metabolic status of the donor hens since Schjeide (1954) has shown dramatic elevations in serum VLDL concentrations with the onset of egg production. LDL concentrations appear rather more stable, however, and several authors including ourselves have reported values close to 100 mg/100 mL serum (Lewis et al., 1952; Fried et al., 1968; Mills and Taylaur, 1971; Hillyard et al., 1972). The levels of HDL in our hens and in those of Mills and Taylaur (1971) were substantially less than the values determined by Hillyard et al. (1972) and by Fried et al. (1968).

On a physicochemical basis, there was an overall resemblance between chicken serum LDL and VLDL and their human counterparts. Some differences were, however, apparent, and these primarily concerned surface charge, chemical composition, and particle size. Thus the low mobility of the chicken lipoproteins upon agarose gel electrophoresis shows them to be of low net surface charge. Furthermore the chicken LDL fraction isolated in a narrow density interval (d 1.024–1.045 g/mL) was larger in diameter (mean 234 Å) than that of the corresponding human fraction (mean 217 Å); this probably results from the twofold higher triglyceride content of the chicken particles, which endows them with a higher molecular weight. The increased triglyceride content was in part compensated by a decreased proportion of cholesteryl ester; the total content of these two lipids was thus essentially identical in the two species (chicken, 46.2%; man, 45.2%). The elevated proportion of triglyceride in the chicken fraction may arise from an increased content of molecular species whose hydrated density is closer to 1.024 than to 1.045 g/mL; this suggestion is consistent with the relatively large amounts of S_f 12–20 substances in our hyperlipoproteinemic birds (Table I).

Although isolated from hypertriglyceridemic laying birds, chicken VLDL were relatively homogeneous in size; VLDL from hyperlipemic human sera tend to be more heterogeneous and to be present over a greater range of particle diameter (e.g., 250–2000 Å, Kane et al., 1975). In view of the direct transfer of VLDL from chicken plasma to egg yolk (Holdsworth et al., 1974), and of the identity of chicken serum and yolk VLDL (Hillyard et al., 1972), it is noteworthy that the egg yolk VLDL fractions isolated by Evans et al. (1975) exhibited similar mean diameters (250, 320, and 390 Å) to those found for serum VLDL in the present study (340 Å).

Our earlier observation of an immunological cross-reactivity amounting to about 10% between chicken and human LDL (Goldstein and Chapman, 1976) was confirmed in the present study with both heterologous and homologous antisera. A similar reaction of partial identity was also detected between chicken and human VLDL. In addition, a common antigen was found in chicken VLDL and LDL, an observation in agreement with the reports of others (Hillyard et al., 1972; Luskey et al., 1974; Hearn and Bensadoun, 1975; Chan et al., 1976). These studies were extended by use of an antiserum to human apolipoprotein B which showed a partial identity between the VLDL and between the LDL of the two species.

Such observations suggested the presence in the avian VLDL and LDL of an apoprotein component resembling apolipoprotein B in man. Comparison of the total apoproteins in the two species showed the solubility properties of chicken apo-VLDL and apo-LDL to resemble those typical of their human equivalents (Shore and Shore, 1972); several characteristics of their amino acid profiles were shared (Table III).

Electrophoresis in sodium dodecyl sulfate-polyacrylamide gel revealed that the major component of both chicken apo-VLDL and apo-LDL was of high molecular weight (>250 000); in this aspect, the patterns were similar to those given by the human fractions. These patterns were confirmed in the electrophoretic system of Takayama et al. (1964); a predominant high-molecular-weight component in the chicken apoproteins was also detected by gel filtration chromatography on Sephadex G-200. Such results are in marked contrast to those of Hillyard et al. (1972), but are, however, in agreement with the data of Hearn and Bensadoun (1975), of Luskey et al. (1974), and with the recent observations of Chan et al. (1976) on chicken VLDL.

Considered together with our own, these data clearly establish the presence of a high-molecular-weight protein(s) as a major component of chicken apo-VLDL and apo-LDL. Its physical properties suggested that it might be the chicken counterpart of apolipoprotein B in man, a supposition confirmed upon its isolation as fraction I by gel filtration chromatography and subsequent characterization. Thus an overall resemblance between the amino acid compositions of fraction I from chicken VLDL and LDL and human apolipoprotein B was apparent (Table IV). Some degree of similarity is also evident upon comparison of the chicken fractions (I) with that of apolipoprotein B from another avian, turkey (Kelley and Alaupovic, 1976). Finally, the behavior of chicken fraction I upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis was essentially identical with that of human apolipoprotein B (Figure 9). We therefore propose to refer to this protein as chicken apolipoprotein B. In view of the studies of Prager and Wilson (1971) on the relationship between immunological cross-reactivity and sequence resemblance among lysozymes, which showed that proteins differing by more than 30–40% in their amino acid sequence fail to cross-react, it seems plausible that human and chicken apolipoprotein B share 60% or more of their sequence in common.

The presence of fast-migrating, low-molecular-weight polypeptides in both chicken VLDL and LDL was indicated by electrophoresis of the total apoproteins in both sodium dodecyl sulfate-polyacrylamide gel and in the system of Takayama et al. (1964). Such components were also detected by Hillyard et al. (1972), by Luskey et al. (1974), and by Hearn and Bensadoun (1975) under similar conditions. Upon gel filtration chromatography, the low-molecular-weight material in fraction II amounted to some 50% of the total protein mass of chicken VLDL, a value akin to that of Chan et al. (1976) (43%). A somewhat similar proportion of soluble apolipoproteins (56%) was detected in human VLDL of mean diameter 367 Å (Kane et al., 1975). The proportion of low-molecular-weight components in chicken LDL of density 1.006–1.063 g/mL was rather less (approximately 15–35%) than in VLDL but greater than in LDL of density 1.024–1.045 g/mL (some 10–25%). The latter chicken LDL therefore contains a larger complement of apolipoproteins of low molecular weight than does the corresponding human fraction (<5%) (Chapman and Goldstein, 1976), an observation which may also result from a skewed distribution of substances with hydrated density close to 1.024 g/mL in this fraction (see earlier Discussion).

Electrophoretic analysis of the soluble apolipoproteins upon treatment of the native chicken lipoproteins with tetramethylurea (Kane, 1973) facilitated the identification of a maximum of seven components (Figure 7); at least three components were present in trace amounts, i.e., <10% on a densitometric basis. Upon comparison with the equivalent pattern from human VLDL, the chicken polypeptides migrated in an area corresponding to that of the more basic human soluble apolipoproteins, i.e., apo-C-I and the arginine-rich peptide (Figure 7). The basic character of the soluble chicken apolipoproteins (from both VLDL and LDL) was confirmed upon amino acid analysis following their isolation as fraction II by gel filtration chromatography (Table IV). Electrophoresis of fraction II from VLDL in 7.5% sodium dodecyl sulfate-polyacrylamide gel consistently revealed three bands of molecular weight 20 300, 13 700, and 10 000; the largest of these may correspond to the apolipoprotein (mol wt about 21 500) claimed by Hillyard et al. (1972) to represent 90% of both chicken VLDL and LDL apoproteins, while one of the smaller components (mol wt 13 700 and 10 000) may correspond to that of 12 000 observed in the low-molecular-weight apoprotein fraction (B) isolated from chicken VLDL by Chan et al. (1976).

Our data concerning the soluble apolipoproteins of chicken VLDL are consistent with those of Chan et al. (1976), although these authors noted only one major band and a further trace component upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis of their low-molecular-weight apoprotein fraction (i.e., fraction B); on reduction, their major component displayed a monomer molecular weight of 6000. As noted above, we consistently detected a greater number of components in sodium dodecyl sulfate-polyacrylamide gel (using 3.3, 7.5, and 10% monomer concentrations) than Chan et al. (1976). Further evidence for the multiplicity of the low-molecular-weight apolipoproteins in chicken VLDL was obtained from studies in the electrophoretic system of Kane (1973), in which at least five components were detectable. Whether these differences emanate from dissimilarities in the size distribution of the respective VLDL populations is indeterminate, since the characteristics of the VLDL species studied by Chan et al. (1976) were not detailed.

It is of considerable interest that Bengtsson and Olivecrona (1976) have recently described the presence of several small polypeptides, ranging in molecular weight from 18 000 to 5000 in chicken egg yolk VLDL. The smallest of these could activate lipoprotein lipase from chicken post-heparin plasma.

In conclusion, the present studies show that VLDL and LDL from hypertriglyceridemic chickens display physicochemical properties in common with their human counterparts, including the presence in their protein moieties of an apolipoprotein B like component. The major difference between the lipoprotein classes of the two species appears to reside in their complement of low-molecular-weight apolipoproteins, which in *Gallus* appears to be rather less complex than in man. Whether such differences bear a relation to the contrasting function of the serum lipoproteins in man and in this egg-laying avian remains to be determined

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